

**EFFECT OF GHEE RESIDUE SUBSTITUTE OF BAKERY
FAT ON QUALITY CHARACTERISTICS OF PEARL
MILLET-BASED BAKERY PRODUCTS**



**THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF**

**MASTER OF TECHNOLOGY
IN
FOOD TECHNOLOGY**

BY

**VINAY G M
B.Tech. (Food Science and Technology)**

**DIVISION OF DAIRY TECHNOLOGY
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE**

**(DEEMED UNIVERSITY)
KARNAL-132001 (HARYANA), INDIA**

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This is to certify that the thesis entitled “Effect of ghee residue substitute of bakery fat on quality characteristics of pearl millet-based bakery products” submitted by Vinay G M towards the partial fulfilment of the requirement for the award of the degree of **Master of Technology in Food Technology** of the **ICAR-National Dairy Research Institute (Deemed University), Karnal (Haryana), India**, is a bonafide research work carried out by him under my supervision and no part of the thesis has been submitted for any other degree or diploma.

Date: 11/8/2022

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निरपेक्षो निर्विकारो निर्भरः शीतलाशयः ।

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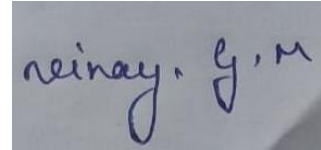
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Vinay G M

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| II | Score Card for Sensory Evaluation of <i>Muffins</i> |

LIST OF ABBREVIATIONS

| | |
|------------------|--------------------------------|
| % | Percent |
| @ | At the rate of |
| °C | Degree centigrade |
| Γ | Gamma |
| µg | Microgram |
| ANOVA | Analysis of variance |
| ATP | Adenosine triphosphate |
| a _w | Water activity |
| Ca ²⁺ | Calcium ions |
| Cm | Cent meter |
| Dl | Deci Litre |
| <i>et al.</i> | Et alii (and others) |
| Fe | Iron |
| G | Gram |
| GR | Ghee residue |
| GRP | Ghee residue powder |
| H | Hour |
| Hp | Horse power |
| Hz | Hertz |
| <i>i.e.,</i> | Id est (in other words) |
| INS | International Numbering System |
| Kg | Kilogram |
| KJ | Kilo joules |
| L | Litre |
| LDPE | Low density polyethylene |
| Ltd | Limited |
| M | Molar |
| Mg | Milli gram |
| Mg ⁺⁺ | Magnesium |
| mL | Milli Litre |

| | |
|-------|--|
| Mm | Milli metre |
| Min | Minutes |
| mT | Million tonnes |
| N | Normality |
| NDRI | National Dairy Research Institute |
| P | Phosphorus |
| Pa | Pascal |
| pH | Negative log of hydrogen ion concentration |
| PMF | Pearl millet flour |
| Ppm | Parts per million |
| Pvt. | Private |
| Rpm | Rotations per minute |
| RWF | Refined wheat flour |
| S. D | Standard deviation |
| Sec | Second |
| SPC | Standard plate count |
| Sp.gr | Specific gravity |
| v/v | Volume/volume |
| w/v | Weight/volume |
| WHO | World Health Organization |
| WPC | Whey protein concentrate |

ABSTRACT

Ghee-residue, obtained after straining molten ghee during its manufacturing is a nutrient-rich by-product of the dairy industry. The majority of ghee residue remained underutilized due to lesser avenues for its commercial application in the food and dairy industry. The ghee residue powder utilised in this investigation had fat ~46.09 %, protein ~31.18 %, lactose ~16.55 %, and ash ~4.91 %. Pearl millet (PM) is considered an important nutraceutical grain due to the presence of a high amount of crude fibre (1.96%), protein (11.14%), ash (1.66%), and fat (5.62 %). Pearl millet is an ideal base material for the manufacture of gluten-free bakery products and attempts have been made to prepare biscuits and muffins. Replacement of ghee residue/ghee residue powder in biscuit dough reduced the lightness, while the redness and yellowness increased. Rheological analysis of dough revealed that it was viscoelastic in nature, with a storage modulus greater than the elastic modulus and a loss tangent less than 0.5, indicating elastic behaviour. Ghee residue utilization lowered dough hardness whereas ghee residue powder addition increased the dough hardness value. Sensory evaluation indicated that bakery fat can be replaced up to the 20 % ghee residue and it was rated best in overall acceptability. The interaction effect of dough resting time and level of baking powder showed that dough resting time of 20 minutes and 0.75 % baking powder was optimum level for developing acceptable quality biscuits. However biscuit sample containing 25 % ghee residue powder, 0.5 % baking powder, and a 30-minute dough resting time resulted in biscuits with maximum overall acceptability score. Hardness and spread ratio of biscuits increased and decreased, respectively with increasing concentration of ghee residue. An attempt was made to prepare eggless muffins by substituting whey protein concentrate with ghee residue powder. Rheological studies revealed that muffin batter displayed shear thinning behaviour and the viscosity was reduced up to ~50%; however further enhancement in GRP level improved the viscosity of batter. The batter's loss tangent was close to 0.8, indicating that the batter was viscous in nature. Increasing the level of Ghee residue powder caused lowering in protein but increase in fat and ash content. Among the physical parameters' redness, specific volume and hardness of muffins increased, whereas lightness value decreased. Based on sensory evaluation, muffin with 25 % WPC substitution with ghee residue powder was acceptable. Replacing more than 50 % of WPC resulted in surface cracking and the collapse of the muffin's body. On the basis of present investigation, it can be inferred that both ghee residue and ghee residue powder can replace fat and WPC in biscuit and muffins respectively without adversely affecting the product characteristics and their sensory acceptability.

सारांश

घी निर्माण के दौरान पिघले हुए घी को छानकर घी-अवशेष प्राप्त किया जाता है, जो डेयरी उद्योग का एक पोषक तत्वों से भरपूर उपोत्पाद है। घी बनाते समय घी अवशेष की पैदावार 2-12% होती है और इसलिए, भारत में बड़ी मात्रा में घी अवशेष का उत्पादन होता है। खाद्य और डेयरी उद्योग में इसके वाणिज्यिक उपयोग कम होने के कारण अधिकांश घी अवशेष का कम उपयोग किया गया है। हालांकि, खाद्य और डेयरी उद्योग में इसके अनुप्रयोग के लिए घी के अवशेष को घी अवशेष पाउडर में बदलने के लिए शोध किया गया है। इस जांच में उपयोग किए गए घी अवशेष पाउडर में वसा (46.09%), प्रोटीन (31.18%), लैक्टोज (16.55%), और राख (4.91%) था। उच्च मात्रा में फाइबर (1.96%), प्रोटीन (11.14%), राख (1.66%), और वसा (5.62%) की उपस्थिति के कारण बाजरा एक महत्वपूर्ण पौष्टिक-औषधीय अनाज माना जाता है। बाजरा ग्लूटेन रहित बेकरी उत्पादों के निर्माण के लिए एक आदर्श आधार सामग्री है और उक्त शोध में ग्लूटेन रहित बिस्कुट और मफिन तैयार करने का प्रयास किया गया है। बिस्कुट के आटे में घी के अवशेष/घी अवशेष पाउडर को बदलने से हल्कापन कम हो गया, जबकि लाली और पीलापन बढ़ गये। आटे के रियोलॉजिकल विश्लेषण से पता चला कि यह इलास्टो-चिपचिपा प्रकृति का था, जिसमें लोचदार मापांक से अधिक भंडारण मापांक और 0.5 से कम का नुकसान स्पर्शरिखा था, जो लोचदार व्यवहार को दर्शाता है। घी अवशेष के उपयोग से आटे की कठोरता कम हो जाती है जबकि घी अवशेष पाउडर मिलाने से आटे कठोरता मूल्य में वृद्धि होती है। संवेदी मूल्यांकन से पता चला कि बेकरी वसा को 20% घी अवशेष तक बदला जा सकता है और इसे समग्र स्वीकार्यता में सर्वश्रेष्ठ दर्जा दिया गया है। आटा नरम करने के समय और बेकिंग पाउडर के स्तर के परस्पर प्रभाव से पता चलता है कि स्वीकार्य गुणवत्ता बिस्कुट विकसित करने के लिए 20 मिनट का आटा नरम समय और 0.75% बेकिंग पाउडर इष्टतम स्तर था। हालांकि बिस्कुट के नमूने में 25% घी अवशेष पाउडर, 0.5% बेकिंग पाउडर और 30 मिनट के आटे के नरम के समय के परिणामस्वरूप अधिकतम समग्र स्वीकार्यता स्कोर वाले बिस्कुट मिले। घी के अवशेषों की बढ़ती सांद्रता के साथ बिस्कुट की कठोरता और प्रसार अनुपात क्रमशः बढ़ा और घटा। व्हे प्रोटीन कॉन्संट्रेट को घी अवशेष पाउडर से प्रतिस्थापित करके अंडा रहित मफिन तैयार करने का प्रयास किया गया। रियोलॉजिकल अध्ययनों से पता चला है कि मफिन बैटर ने कतरनी पतले व्यवहार को प्रदर्शित किया और चिपचिपापन ~ 50% तक कम हो गया; हालांकि घी अवशेष पाउडर स्तर में और वृद्धि से घोल की चिपचिपाहट में सुधार हुआ। घोल का नुकसान स्पर्शरिखा 0.8 के करीब था, यह दर्शाता है कि घोल प्रकृति में चिपचिपा था। घी अवशेष पाउडर का स्तर बढ़ने से प्रोटीन में कमी आई लेकिन वसा और राख की मात्रा में वृद्धि हुई। भौतिक मापदंडों में लालिमा, विशिष्ट मात्रा और मफिन की कठोरता में वृद्धि हुई, जबकि लपट मूल्य में कमी आई। संवेदी मूल्यांकन के आधार पर, घी अवशेष पाउडर के साथ 25% डब्ल्यूपीसी प्रतिस्थापन के साथ मफिन स्वीकार्य था। 50% से अधिक डब्ल्यूपीसी को बदलने से मफिन में दरार आ गई। वर्तमान जांच के आधार पर, यह अनुमान लगाया जा सकता है कि घी अवशेष और घी अवशेष पाउडर दोनों उत्पाद विशेषताओं और उनकी संवेदी स्वीकार्यता पर प्रतिकूल प्रभाव डाले बिना क्रमशः बिस्कुट और मफिन में वसा और डब्ल्यूपीसी की प्रतिष्ठापित कर सकते हैं।



CHAPTER 1

INTRODUCTION

Introduction

Bakery products constitute an important component in our diet because of the convenience, availability in different forms, unique sensory profile and availability at a relatively lower price. There is growing demand of bakery products both for home consumption and at foodservice industry. Among the bakery products biscuits, cookies and crackers are preferred by people of all age groups and economic segments as they are self-stable and nutrient dense. Furthermore, the bakery industry is continually innovating for the creation of newer variants using different formulations, additives, novel ingredients, flavour; machinery and packaging. Diversification has resulted in a further upsurge in their consumption and market growth. Rapid economic expansion, the establishment of food, chains and changing eating habits have all contributed to a large increase in popularity among the common people.

It is projected that during the period 2021-2028, the worldwide foodservice market is expected to grow at a CAGR of 10.34 %, from USD 2,525.4 billion in 2021 to USD 5,027.9 billion in 2028 (Anonymous, 2022). The global bakery products market is expected to increase at a CAGR of 5.12% from USD 416.36 billion in 2021 to USD 590.54 billion by 2028 (Anonymous, 2022). In 2020, the Indian bakery market was valued at approximately USD 7.60 billion which is estimate to reach figure of USD 12.39 billion with expected growth rate of 8.5%. However, considering the higher calorie density owing to higher levels of both fat and sugar, the trend is towards healthier bakery products such as low sugar, trans-fat free, high protein, high fiber and low-fat ones. These trends are further fuelled by urbanization, increasing trends for eating out, health consciousness and demand for newer products. In India, the biscuit and cookies business constitutes approximately 72 % of the nation's total bakery sales. India is currently the world's largest biscuit consumer, with a market value of about USD 4.65 billion in FY 2020 (Anonymous, 2021). A trend toward most delicate biscuits such as sugar-free, oats, wheat, and multi-grain biscuits, among others, is escalating the market's rise.

Cereals mainly wheat flour is the major raw material used for the manufacturing of bakery products. Cereals are the major staple in our diet across the globe and an important source of calories, proteins, minerals and vitamins, especially the vitamin B group. The contribution of cereals in meeting food and nutritional security is unparalleled at the global level. In 2019, on average 2818 million tonnes of cereals were produced globally. Estimated coarse cereal production in India is around 47.48 MT in the year 2019-20 which showed a CAGR growth rate of 1.39% (FAO STAT, 2021). With limited land, water resources, climate and

growing population issues have been raised regarding the sustainability of enhanced cereal production is remain a daunting task. Millets being hardy, and able to grow even under harsh-dry and scanty rainfall are considered crops of the future in our pursuit of ensuring nutritious foods to the ever-growing population on earth.

Millets are growing and consumed extensively in semi-arid areas of Africa and Asia (India & China). As per FAO STAT 2020 millets are the sixth-highest yielding grains. Total millet global production was 28.37 million tonnes in 2019; of which Asia produces nearly 48% followed by Africa (47.9%), Europe (2.9%) & America (1%). India is the largest producer of millet with 12.66 MT in 2019 with a CAGR of 1.83% followed by Niger & China.

Wheat is one of the most often used grains in the world as a food ingredient. Wheat is the major raw ingredient for baked products because of the presence of gluten proteins that impart characteristic visco-elastic nature (Puniya *et al.*, 2021). Gluten proteins are present in lower amounts in maize, barley, and oats but absent in millets. In the recent past with the emergence of celiac diseases that cause inflammation of the intestinal wall, there is a need for alternatives which can replace wheat and gluten-containing grains in diet (Rona *et al.*, 2007). In celiac diseases gluten causes distinctive damage to the small intestinal mucosa in genetically sensitive patients (Hadimani *et al.*, 1995). Millets are rich in fibres, micronutrients such as iron, zinc, copper, vitamins B group, polyphenols and a wide variety of phytochemicals. The starch of millet crops is also slowly digestible and the presence of enzyme inhibitors lowered the starch digestion in the gut which makes them an ideal dietary component for diabetes and obese individuals. Higher levels of anti-oxidants in millets assist in minimizing oxidative stress and thus lifestyle associated diseased (Nani *et al.*, 2015). Pearl millet has been attempted by a number of researchers as an alternative to developing gluten-free processed products because of its unique physicochemical, nutritional and techno-functional properties besides its easier availability (Jukanti *et al.*, 2016). Pearl millet is an ideal base material for the manufacturing of gluten-free bakery products and attempts have been made to prepare shelf-stable flour, biscuits, bread, pasta, extruded snacks, composite beverage, complementary foods and modified starches using pearl millet (Yadav *et al.*, 2012; Sharma *et al.*, 2016; Adebisi *et al.*, 2017; Kumar *et al.*, 2019; Ganguly *et al.*, 2019 and Ganguly *et al.*, 2021).

However, pearl millet-based products are criticized for their poor colour, off- flavour and inferior textural attributes which can be overcome by appropriate formulation and incorporation of novel additives Certain processing interventions and selection of appropriate

varieties of pearl millet could be effective strategies to develop acceptable quality food products with unique health properties.

Ghee is one of the major indigenous fat-rich dairy products of India which is manufactured at various levels of production. With an estimated production of about 1,70,000 MT India is the largest producer of ghee and its recent growth rate CAGR of 3.60% (Anonymous, 2022). During the manufacture of ghee, a nutrient-rich brown/dark coloured residue mainly consists of solid-not-fat (SNF) fraction along with entrapped milk fat. The amount of ghee residue obtained during the process varies depending on the raw material used for ghee manufacturing and the method of preparation, but usually, on average, the yield is about 10 % (Verma and Raju, 2008). There are three different methods of ghee manufacture and all these processes result in the generation of residue that differs in composition and other parameters depending on the method involved in its production.

Ghee-residue is a by-product which is moist brownish sediment obtained after straining of molten ghee during its manufacturing (Janghu *et al.*, 2014). It is nutrient-dense and contains 36-65 % fat, 18-42 % protein, 8-30 % moisture, 0.5-15 % lactose and 2-5 % ash (Ramesh *et al.*, 2018). Ghee residue is high in milk nutrients and can act as a potential antioxidant and flavouring ingredient in food products (Wadhwa *et al.*, 1990). A significant amount of ghee residue is discarded due to a lack of profitability or lack of knowledge regarding the options that can be employed by the industry to harness the nutritional and therapeutic properties of ghee residue. Wani *et al.* (2022) published an exhaustive review based on scientific publications encompassing the various aspects of ghee residues. Authors are of the opinion that compositional features, excellent antioxidant properties, and higher levels of denatured proteins make it a promising ingredient in certain processed foods. Hence, technology must be developed, and/or emphasis must be given to the development of snacks based on ghee residue, which would provide a wide range of nutritional possibilities as well as product diversity, resulting in increased industry productivity and lower organic load in the effluent treatment plant. However, dark colour, cooked flavour and higher levels of cooked particles limit their application to the only bakery; confectionary and heat desiccated dairy products. Attempts have been made in our laboratory to convert ghee residue into powdered form by blending it with water or skim milk or buttermilk. The developed powders termed ghee-residue powder (GRP) possess excellent techno-functional properties which could be exploited in the formulation of a wide range of processed products.

In bakery products, ghee-residue incorporation may provide additional fat and proteins besides offering phospholipids which exhibit excellent emulsification and nutritional properties. Moreover, Maillard reaction products present in ghee residue and ghee residue powder mimic the colour of cocoa powder and may act as anti-oxidants as well. It is conceptualized that incorporation or partial substitution of ghee-residue or ghee residue powder as fat or protein replacer in pearl millet and wheat flour-based composite formulation could improve the sensory, textural and nutritional properties of the resultant products. However, a systematic investigation is required to ascertain the effect of substitution of bakery fat with ghee residue on dough/batter rheology, physicochemical, textural, nutritional and sensory quality of biscuits and muffins. Keeping the above-mentioned points under consideration, the present research investigation is undertaken with the following objectives:

1. Effect of ghee-residue or ghee-residue powder (GRP) on rheological and physicochemical properties of biscuit dough and muffin batter
2. Standardisation of ghee residue ghee-residue powder (GRP) level in the formulation of pearl millet-based biscuit and muffins



CHAPTER 2

REVIEW OF LITERATURE

Review of literature

The chapter provides information related to the proposed research area including the previous work carried out, major findings; the genesis of the problem; researchable issues and work that still needs to be done systematically. Attempts have been made to compile the available scientific and technical literature available on pearl millet: its importance, nutrition value, health benefits and technological challenges in utilization of pearl millet in bakery products: biscuits & muffins and ghee residue: definition, potential flavour properties of ghee residue, nutritional composition, antioxidant properties, application in food & non-food and technological development to improve the functional properties. This chapter includes the following major sections:

2.1 Pearl millet

2.2 Biscuits & muffins

2.3 Ghee residue

2.1 Pearl millet

Pearl millet (*Pennisetum glaucum*) is a multipurpose cereal grown for food, feed, and fodder, especially in African and Asian countries (Arora *et al.*, 2003). Pearl millet, also known as bulrush millet, is amongst the most drought-tolerant cereal which can withstand physiological stresses such as high temperature, high salinity & drought (Rai *et al.*, 2008). Pearl millet is grown as a rain-fed crop in Southern Africa & India's semiarid regions, where the total annual precipitation varies from 200 to 800 mm; the average yearly temperature is higher and is often drought-prone. In India, the crop is extensively grown in Rajasthan, Maharashtra, Telangana, Gujrat, Andhra Pradesh, Karnataka, Uttar Pradesh and Haryana. Rajasthan is the leading producer of pearl millet producing around 4.69 MT followed by Uttar Pradesh (~1.94 MT) (Directorate of Economics & Statistics, 2021). Pearl millet is known as "Nutri-cereal" because of its high fibre, protein, fat (rich in unsaturated fatty acids) and mineral composition. Protein makes up of pearl millet lack gluten proteins which makes it an ideal alternative food for celiac as well as gluten-intolerance people (Rona *et al.*, 2007). As a food crop pearl millet is consumed by the local ethnic population as a staple but the presence of thick pericarp, extensive pigmentation, higher levels of phenolics related to bitterness or astringency, highly active lipase and higher concentration of anti-

nutrients such as phytates, enzyme inhibitors, oxalates and polyphenols, are the major obstacle in their widespread consumption.

2.1.1 Importance of pearl millet

Pearl millet is one of the most nutritious cereal grains and can be compared with major staples i.e., wheat, rice and maize. The approximate per cent of endosperm, germ, and bran in pearl millet grains are 75%, 17%, and 8%, respectively (Satankar *et al.*, 2020). The protein level and quality of protein are comparable to conventional cereals. Further, average lysine content is slightly higher i.e., ~3.68 mg/gram of protein, compared to ~2.24 for wheat, ~3.36 for rice, ~3.0 for maize, and ~3.2 for sorghum (Saleh *et al.*, 2013). The carbohydrate content is comparably higher (67–72%), with starch being the most important constituent and the remaining carbohydrates are non-digestible ones which assist in improving gut health. The fat content that varies in the range of 4–8% is also higher in comparison to other cereals and may be one of the factors for their higher acceptability and palatability (Amadou *et al.*, 2013). The ash content ranges from 1.5 to 2.7%, and the phosphorous content is high (270–390 ppm) (Krishnan & Meera, 2018). In addition, the grain is also rich in vitamins, particularly thiamine, riboflavin, and nicotinic acid. In the area of their production, pearl millet contributes significantly to ensuring food and nutritional security as most of the population harbouring these areas is poor.

2.1.2 Nutrition value of Pearl Millet

The detailed chemical composition of the pearl millet, other millets and cereals are given in Table 2.1. It can be observed from a composition that pearl millet/bajra is superior to all other millets, and it is evenly comparable with the cereals. There is a growing trend of consuming whole grain-based products chiefly due to their richness in dietary fibre, vitamins, and nutraceuticals mainly antioxidants (Gong *et al.*, 2018). Pearl millet has a better nutritional characteristic in terms of carbohydrates, dietary fibre, proteins with balanced amino acid composition, numerous essential minerals, certain vitamins, and antioxidants. These elements play a significant role in the prevention of rapidly growing lifestyle-associated diseases such as diabetes, cancer, cardiovascular, and neurological disorders (Jukanti *et al.*, 2016). These diseases are attributed to a sedentary lifestyle, consumption of refined and highly processed food products, lower consumption of fibre-rich foods, lower intake of anti-oxidants in diet and a diet rich in saturated fats.

2.1.2.1 Carbohydrate

The carbohydrate content of pearl millet is around 60-62%, which is lower than the other cereals such as rice (~78.24%), wheat (~64.17%) and maize (~64.77%). The diameter of pearl millet starch granules varies from 3.5 to 23 μm (Annor *et al.*, 2014). Starch granules have a pore-deep surface, are polygonal and spherical in shape, and differ in size from small to large (Sandhu *et al.*, 2017). Amylose content ranges from 21.9 to 28.8% which is similar to other cereals and the soluble sugars are in the range of 1.2 to 2.6%. Suma *et al.* (2015) reported that MRB and K varieties of pearl millet have lower levels of starch i.e., 34.5 and 39.4 g/100 g respectively. Pearl millet starch also exhibits functional properties like thickening, gelling, and swelling which has a profound effect on the texture of processed products (Hassan *et al.*, 2021). The average total dietary fibre content is 11.50% which is significantly higher than rice, sorghum and other food grains. The dietary fiber content of PM is lower than coarse cereals such as barley, oat and maize. Consumption of fibre-rich foods is usually recommended because of their positive effect on gut health, blood lipid and cholesterol level that reduces the risk of developing inflammatory bowel disorders and cardiovascular issues (Barber *et al.*, 2020).

Table 2.1: Proximate composition of millets, coarse cereals & fine cereals (per 100 g)

| Name | Moisture (g) | Protein (g) | Ash (g) | Total Fat(g) | Dietary fibre | | | Carbo-Hydrate | Energy (KJ) |
|----------------|--------------|-------------|---------|--------------|---------------|-----------|---------|---------------|-------------|
| | | | | | Total | Insoluble | Soluble | | |
| Millets | | | | | | | | | |
| Pearl | 8.97 | 11.84 | 1.37 | 5.43 | 11.49 | 9.14 | 2.37 | 61.78 | 1456 |
| Finger | 10.89 | 7.16 | 2.03 | 1.92 | 11.18 | 9.51 | 1.67 | 66.82 | 1342 |
| Porso | 11.36 | 10.13 | 1.34 | 3.89 | 7.72 | 5.45 | 2.27 | 65.55 | 1449 |
| Foxtail | 14.23 | 8.92 | 1.72 | 2.55 | 6.39 | 4.29 | 2.11 | 66.19 | 1388 |
| Kodo | 9.87 | 8.3 | 3.6 | 1.4 | 2.83 | 1.71 | 1.12 | 72.00 | 1456 |
| Little | 11.2 | 8.7 | 4.3 | 4.7 | 8.7 | 7.99 | 0.71 | 60.9 | 1490 |
| Barnyard | 11.27 | 11.6 | 4.2 | 4.8 | 6.43 | 4.81 | 1.62 | 60.3 | 1463 |
| Cereals | | | | | | | | | |
| Barley | 9.77 | 11.5 | 1.06 | 1.30 | 15.64 | 9.98 | 5.66 | 61.29 | 1321 |
| Sorghum | 9.01 | 9.97 | 1.39 | 1.73 | 10.22 | 8.49 | 1.73 | 67.68 | 1398 |
| Maize | 9.26 | 8.80 | 1.17 | 3.77 | 12.24 | 11.29 | 0.94 | 64.77 | 1398 |
| Wheat | 11.0 | 10.57 | 1.28 | 1.53 | 11.36 | 9.73 | 1.63 | 64.17 | 1340 |
| Rice | 9.93 | 7.94 | 0.56 | 0.52 | 2.81 | 1.99 | 0.82 | 78.24 | 1491 |

(Sokrab *et al.*, 2012; Longvah *et al.*, 2017)

Table 2.2: Amino acid profile of pearl millet and wheat

| Amino acid (g/100 g) | Pearl millet | Wheat |
|-----------------------------------|--------------|--------------|
| Essential amino acid | | |
| Methionine | 1.00 | 1.5 |
| Leucine | 14.1 | 7.0 |
| Isoleucine | 5.1 | 3.7 |
| Valine | 4.2 | 4.4 |
| Phenylalanine | 7.6 | 4.9 |
| Tryptophan | 1.2 | 0.16 |
| Lysine | 0.5 | 2.7 |
| Threonine | 3.3 | 2.7 |
| Histidine | 1.7 | 2.4 |
| Total essential amino acid | 38.7 | 29.46 |
| Nonessential amino acid | | |
| Proline | 8.2 | 11 |
| Tyrosine | 2.7 | 1.4 |
| Glycine | 0.7 | 3.5 |
| Alanine | 8.1 | 3.3 |
| Aspartic acid | 6.2 | 4.4 |
| Glutamic acid | 22.8 | 32.1 |
| Tyrosine | 2.7 | 1.4 |
| Arginine | 0.9 | 3.7 |
| serine | 5.4 | 4.5 |
| Cystine | 0.8 | 0.68 |

(Shoup *et al.*, 1966; Amadou *et al.*, 2013)

2.1.2.2 Protein

The second most abundant content in pearl millet is protein after carbohydrate, which varies in the range of 9-11.5% and it is similar to wheat, barley and barnyard millet. The amino acid profile of the pearl millet and wheat is depicted in Table 2.2. It can be observed that the essential amino acids particularly, methionine, valine, histidine, glycine and arginine are significantly higher in wheat but the concentration of remaining amino acids is higher in pearl millet. Pearl millet is rich in lysine (22.8 g/100 g of protein) which is lower than wheat

(32.1 g/ 100 g protein). Glutamic acid is a non-essential amino acid, which is a precursor of γ -amino butyric acid (GABA), an essential component for neuro-transmission (Han *et al.*, 2015). Glutamic acid helps in the transport of K^+ ions through the blood-brain barrier and removes the brain ammonia by attaching it to nitrogen atoms in the brain. Cohort studies also indicated that glutamic acid has an active role in cognitive functions such as memory consolidation in the brain, despite the risk of neuronal damage associated with diseases such as amyotrophic lateral sclerosis, lathyrism, and Alzheimer's disease at higher levels (Dutta *et al.*, 2011). It was also observed that glutamic acid is also involved in the prevention and combating of cancer cells (Dutta *et al.*, 2013) and that the essential amino acid levels of pearl millets are higher than in wheat (Table 2.2) where the total essential amino acid concentration in pearl millet is much higher than wheat (~38.7 g and ~29.46 g/100 g of protein respectively).

2.1.2.3 Lipid

The lipid content of pearl millet varies between 5-7% due to the presence of germ which is rich in lipid content (Gopalan *et al.*, 2003). Higher lipid content in pearl millet flours renders it unstable and prone to oxidation as lipids in PM are rich in unsaturated fatty acids (Tiwari *et al.*, 2014). Further, pearl millet has a higher proportion of fatty acids particularly linoleic (39-42%), stearic (26-28%), and palmitic acids (20-21%) whereas MUFA levels are low (Adeola & Orban, 1995). Pearl millet grains contain active lipase and size reduction operation such as milling diminishes the protective barrier and both substrates as well as enzymes, interact with each other leading to lipolytic rancidity. The development of off-flavour and bitterness is the major reason for the non-availability of primary processed products from millets.

2.1.2.4 Minerals

The mineral content measured as ash in pearl millet is about 1.35-1.45% which is slightly higher than wheat (1.28%) and other cereals. In contrast, it is lower than the finger millet (2.03%) as the latter contains exceptionally higher levels of calcium. The different minerals reported in pearl millet are as follows: remarkably higher content of phosphorous (296 mg), potassium (393 mg), and magnesium (133 mg) but lower levels of calcium (42 mg) and iron (4.1 mg) when compared to wheat and other cereals (Ragae *et al.*, 2006). The higher levels of phosphorous in pearl millet can be significantly correlated to its importance in bone formation, for maintaining several metabolic activities (like ATP formation) and

hormonal regulation (Wood, 2006). Likewise higher potassium and magnesium content are considered heart healthy.

Table 2.3: Average chemical compositional values of various types of millet and cereals (per 100g) and their glycemic index

| Name | Ca ²⁺ (mg) | P (mg) | Fe (mg) | Thiamine (mg) | Niacin (mg) | Total phenol (mg/100 g) | Glycemic index |
|----------------|--------------------------|-----------|------------|------------------|----------------|----------------------------|-------------------|
| Millets | | | | | | | |
| Finger | 344 | 283 | 3.9 | 0.42 | 0.19 | 102 | 49.17 |
| Porso | 14 | 206 | 0.8 | 0.2 | 0.18 | 0.10 | 53.79 |
| Pearl | 42 | 296 | 4.1 | 0.33 | 0.25 | 51.4 | 54.15 |
| Foxtail | 31 | 290 | 2.8 | 0.59 | 0.11 | 106 | 47.54 |
| Kodo | 27 | 188 | 0.5 | 0.33 | 0.09 | 368 | 41.43 |
| Little | 17 | 220 | 9.3 | 0.30 | 0.09 | 21.2 | 44.53 |
| Barnyard | 14 | 121 | 5.0 | 0.33 | 0.1 | 26.7 | 49.05 |
| Cereals | | | | | | | |
| Barley | 26 | 215 | 0.25 | 0.19 | 0.11 | 16.4 | 68.7 |
| Sorghum | 25 | 222 | 8.0 | 0.33 | 0.10 | 43.1 | - |
| Maize | 20 | 348 | 2.1 | 0.15 | 0.05 | 2.91 | 84.7 |
| Wheat | 41 | 306 | 5.3 | 0.45 | 0.17 | 20.5 | 64.25 |
| Rice | 10 | 160 | 0.7 | 0.06 | 0.06 | 2.51 | 82.57 |

(Gopalan *et al.*, 2009; Prasad *et al.*, 2015 & Bijlani *et al.*, 1991)

2.1.2.5 Bioactive compounds in Pearl millet

The pearl millet contains a considerable amount of fat-soluble vitamins, phenolics and a few other phytochemicals. The average vitamin and total phenolic content of various millets and cereals are presented in Table 2.3 (Hassan *et al.*, 2021). Pearl millet is rich in certain vitamins such as vitamin E (2 mg/100g), thiamine (0.33 mg/100g) & niacin (0.25 mg/100g), however, the thiamine content of wheat is higher than the pearl millet. It is depicted from the table that the total phenolic component in the pearl millet is higher and responsible for potentially higher antioxidant properties (N'Dri *et al.*, 2013). Millets have been identified as a better source of vitamins and phenols than staple cereals. The presence of polyphenols and dietary fibres provide an additional benefit of functional and nutraceutical property to a food. Flavonoid present in certain millet is known to possess antioxidant and anti-inflammatory activities (Shahidi & Chandrasekara, 2013).

Generally, millets are consumed as whole grain rather than the only endosperm portion that primarily contains starch. The antioxidant property of millets is mainly credited to the presence of polyphenols, flavonoids, and other phytochemicals which are mainly concentrated in the bran fraction (Chandrasekara & Shahidi, 2010). The polyphenols like

phenolic acid and tannins are known to form complexes with proteins and particularly with enzymes like amylases associated with starch digestion; thus, slowing down their digestion. It has been observed that these components inhibit the activity of α -amylase, pancreatic amylase, and glucosidase enzymes, thereby checking postprandial hyperglycaemia (Thilagavathi *et al.*, 2015). Furthermore, polyphenols play a crucial role as anti-inflammatory, antioxidant and antiviral compounds. Another study reported that ferulic acid and *p*-coumaric acid present in the pearl millet have the ability to reduce cancerous cell progression (Sarita & Singh, 2016). However, the presence of tannins interrupts the absorption of protein and minerals (Chavan *et al.*, 2001). Another *in-vitro* study conducted by Radhajeyalakshmi *et al.* (2003) reported that the proteins of pearl millet effectively inhibit the growth of phytopathogenic fungi like *Fusarium oxysporum*, *Macrophominaphaseolina*, and *Rhizoctonia solani*. Based on the above-mentioned discussions it can be inferred that bioactive components present in pearl millet have the potential to address a number of health issues including cardiovascular diseases, cancer, diabetes and immune disorders.

2.1.3 Health benefits of pearl millet

Diabetes was responsible for 1.5 million deaths in 2019 out of which 48% of mortality was among people aged less than 70 years. (WHO, 2021). A number of factors responsible for diabetes include consumption of food having a high glycemic index or higher calorific values obesity, genetics and a sedentary lifestyle. The richness of pearl millet in dietary fibres assists in minimizing the cholesterol absorption in the gut, slowing down starch digestion through physical entrapment, bulking and efficient transition of stool through the GI tract. Moreover, fibres also act as prebiotic substances and colonic fermentation leads to the production of short-chain fatty acids (SCFA) which play a significant role in enhancing gut health (Sharma & Kapoor, 1996). Sukar *et al.* (2020) studied that feeding of pearl millet (20%) to control and diabetic rats manifested in an increase in the insulin concentration significantly from 0.97 μ /ml to 1.04 μ /ml, lowering the low-density lipoprotein from 45 mg/dl to 40 mg/dl and reduction in blood glucose level in the diabetic rat from 203 mg/dl to 129 mg/dl.

Blood glucose level depends on the digestibility of starch which basically depends on the type and form of starch. These starches are classified into three types, namely slow-digestible starch, rapidly-digestible starch, and resistant starch, which is not hydrolysed by enzymes in the small intestine and instead reaches the large intestine. Resistant starches are metabolized by colonic bacteria and produce a number of compounds. Rapidly digestible

starch is hydrolysed and assimilated quickly, resulting in a rapid rise in blood glucose levels and thereby contributing to hyperglycaemia, obesity, diabetes and heart diseases (Chai *et al.*, 2013). Since millet starches contain a higher proportion of long chains of amylose and prolonged chain segments between branch points, enzymatic starch hydrolysis and glycaemic index are anticipated to be low in millet starches (Annor *et al.*, 2013). The glycemic index of various millets and cereals is presented in Table 2.3. It is evident that the glycemic index of pearl millet was less (54.15) compared to other cereals and it may be attributed to trans-cinnamic acid, a phenol present in millets which inhibits amylolytic enzymes (Rohn *et al.*, 2002). According to WHO (2022) report, one in six deaths, or 9.6 million deaths, was attributed to cancer in 2018, making it the second largest cause of death worldwide. Cancer is caused by a number of factors like smoking, exposure to radiation, pesticides, chronic inflammation and genetic mutation etc. According to one report, about 70% of cancers are related to diet and elimination of carcinogenic substances from the diet is not possible. Studies showed that the choice of food that is consumed during life is one of the major causative factors for various lifestyle diseases (Shan *et al.*, 2015). Free radicals, reactive oxygen species (ROS) and free-radical mediated compounds are known to initiate cancer cell formation. The anti-oxidant theory postulates that lower consumption of anti-oxidant rich foods is the major reason for increasing incidences of cancers, coronary heart diseases, arthritis and diabetes. The phenolics and tannins in pearl millet prevented the formation of tumour cells (Huang and Ferraro, 1992).

Ganguly *et al.* (2019) studied the effect of probiotic beverages on *shigella*. The beverage was prepared by fermenting a composite of whey & skim milk mix (60:40), pearl millet (~4.73%) and barley (~3.27%) with probiotic bacteria. Animal investigations showed that the beverage effectively controlled the shigella and also increased the IgA in the intestinal fluid. In-vitro fermentation using probiotic strains of *Lactobacillus acidophilus*, *Lactocaseibacillus rhamnosus*, *Bifidiobacterium bifidus*, and *Bifidiobacterium longum* of millet dietary fibre fractions, resulted in the synthesis of short-chain fatty acids like acetate, propionate, and butyrate at elevated levels (Arora *et al.*, 2011). Millets are a good source of magnesium, which has been shown to lessen the symptoms of headaches and cardiac arrest (Sarita & Singh, 2016).

2.1.4 Technological challenges

Millets are still used only in rural areas and low-income families; this is due to a lack of technology in millet processing and difficulty in decortication. This limits the conversion

of millet into ready-to-eat, ready-to-cook & easy to handle products at the commercial level. Generally, bitterness and rancidity are developed when pearl millet flour is stored and thereby reducing its shelf life. These problems are mainly attributed to the activity of the lipase enzyme, which causes the breakdown of glycerides and an increase in the profile of free fatty acids (Arora *et al.*, 2002). Therefore, inactivation before milling is necessary to enhance grain quality and improve shelf life. The pearl millet grain can be given a dry heat treatment to achieve this, which suppresses lipase activity and down-surge lipid breakdown during storage (Rai *et al.*, 2008).

Kadlag *et al.* (1995) revealed that dry heat treatment for 120 minutes to pearl millet grains caused a significant decrease in fat acidity, acid value, and free fatty acid profile throughout the length of a 30-day storage period. Before milling, pearl millet grains were dry heated, which enhanced flour quality while having no appreciable impact on acceptance. According to Nithya *et al.* (2007) certain anti-nutritional compounds like polyphenols (from 3.00 to 2.27 g/100 g) and tannin (from 1.52 to 1.30 g/100 g) were dramatically reduced when whole pearl millet grains were subjected to a dry heat treatment (110 °C/1 h).

Pearl millet contains anti-nutritional factors like phytic acid, C-glycosylflavones (C-GFs), or goitrogenic polyphenols which include glucosylvitexin, glucosylorientin, vitexin and enzyme inhibitors (Dias-Martins *et al.*, 2018). They interfere in the absorption of macro & micronutrients. Hence, it may cause health problems and affect the acceptability of millets at the consumer end. (Rani *et al.*, 2018). Dehulling, fermentation, malting, acid treatment and heat treatments etc, methods can be used to reduce the anti-nutritional factors (Dias-Martins *et al.*, 2018).

2.1.5 Utilization of pearl millet

Millet pearl grains are used to make a wide variety of traditional local foods in Africa and India, including porridges, dumplings, flatbreads, couscous, sweets, alcoholic beverages (namely, Boza, Merrisa & Chapalo) and non-alcoholic beverages (namely, Kunu-Zaki, Oshikundu & Bushera) (Adebiyi *et al.*, 2016).

Ghanaian Koko: It is a millet porridge that is consumed on a daily basis as an in-between meal or snack (Muller, 1970). It is produced by steeping bajra overnight and grounded to make a slurry out of it. The slurry is then sieved and allowed for fermentation and sedimentation. The top water is then boiled, and the sedimented bottom layer is added to achieve the desired consistency (Lei and Jakobsen, 2004).

Porridge (Koko, ugali): Porridges can be made from fermented or unfermented pearl millet flour. Depending on the amount of flour used, their consistency can range from thick to thin (30 %- 10 %). Cooking flour in boiling water with vigorous stirring allows you to make a variety of porridges. Furthermore, the flavour and pH of these products can vary greatly depending on the added ingredients like lemon juice & tamarind extract etc (Kajuna, 2001).

Fura: It is a staple food in Nigeria, Ghana, and Burkina Faso. It is a traditional semi-solid dumpling made by steaming fermented moist pearl millet flour balls, accompanied by smashing in a mortar and rolling (Owusu-Kwarteng *et al.*, 2010). It can be consumed along with *dahi/yoghurt*.

Flatbread: In other words, chapati, if it's made from fermented flour called lohoh (Saudi Arabia) otherwise roti (India). These flatbreads are made by combining bajra flour and warm water, then cooking them on a hot plate (Tawa), a clay griddle, or a wood fire stove and serving them with hot pickles or spicy sauces, depending on the region.

Puffing/popping: Popped finger millet is a whole-grain popped ready-to-eat product rich in macronutrients, micronutrients, dietary fibre and is usually mixed with milk or vegetable protein sources such as popped chickpea, milk powder, and oil seeds, sweetened with sugar or jaggery to prepare ready-to-eat nutritious supplementary food (Malleshi, 2007). Similarly, bajra was popped by maintaining a moisture content of 19 % and popped at 270 °C. This popped bajra is also used to make laddoo (Singh, 2008)

Kunu-Zaki: It's a Nigerian non-alcoholic fermented beverage. Grains are washed, dried, and ground coarsely. To obtain the product, the flour is mixed with hot water to form a paste, which is then spontaneously fermented for days. Ginger is also added to enhance the sensorial qualities (Efiuvwevwere & Akona, 1995).

The use of novel starter bacteria with amylolytic, proteolytic, and phytase activity has been developed for pearl millet (Bajra) lassi, and it has been shown to improve the lassi's shelf life (Chakraborty & Chakraborty, 2021). Ganguly *et al.* (2021) developed a probiotic beverage from underutilized sources like whey along with the incorporation of pearl millet and barley, it was fermented with *Lactobacillus acidophilus*. *In-vivo* studies reveal that protein digestibility was improved and the mice gained weight better than the control group and the blood haemoglobin level was also improved. Bunkar *et al.* (2014) developed an instant kheer mix using pearl millet, dairy whitener and sugar.

2.2 Biscuits and Muffin

Bakery goods are getting more and more popular every day due to their enhanced nutritional content and suitability as food in crises, such as earthquakes. There has been a lot of research done on cereal science and technology, which gives us a good idea of the research that has been done on bakery products (Arepally *et al.*, 2020). Biscuits, among other bakery items, are a versatile snack in the food industry due to their enticing properties, including longer shelf life, a diversity of flavours and textures, and broad consumption (Nagi *et al.*, 2012).

2.2.1 Biscuit

The term "biscuit" comes from the Latin "*panisbiscoctus*", which denoted the twice-cooked bread popular among sailors and known as "ship biscuits". Refined wheat flour is widely used to manufacture biscuits despite having a very low nutritional value due to its high gluten content (Agrahar-Murugkar *et al.*, 2015). Therefore, it is necessary to find alternative flours that can partially or entirely replace refined flour in bakery items. Apart from this, the biscuits were manufactured with high fat and sugar content (Moriani *et al.*, 2018). Since fat is responsible for the flavour, texture, and aesthetic quality of the finished product, reducing and replacing fat is a major challenge for researchers.

Definition:

FSSAI defined a biscuit as a,

(i) Biscuit is a baked product leavened or non-leavened, coated or uncoated, centre-filled partially or wholly such as but not limited to wafer biscuits, coated wafers, cookies, crackers, centre-filled biscuits, enrobed biscuits, sandwich biscuits, cream biscuit including fat-free/ low fat or sugar-free/ low sugar variants.

(ii) It can be made from cereal and cereal products including millets, pulses, legumes and mixtures. It may also contain fats and oils, including fat emulsions etc. or mixture thereof, Baking powder, sugar and sugar products, edible common salt including salt substitutes, dairy products and analogues, nutritive and non-nutritive sweeteners, honey, invert sugar, jaggery, dextrose, edible molasses, liquid glucose/glucose syrup (High Maltose/High fructose), fruits and vegetables and their products (including dried fruits and vegetables), cocoa and its products including chocolates, tea, coffee, chicory and their extracts, coconut and its products, eggs and egg products, gluten, nut and nut products, malt and malt products, milk and milk products, oilseeds and its products including oilseed flours, all edible starches

and edible flours, spices, condiments, herbs and their extracts, seasonings, vinegar, edible seeds, protein concentrates/isolates, Yeast and its products including yeast extract, enzymes, nutrients like vitamins and minerals, edible fibres, maltodextrin, oligofructose, trehalose and any other ingredients as specified in Food Safety and Standards Regulations.

(iii) It shall conform to the following requirements, namely: -

| Requirements | Limits |
|---|--------------------|
| Ash insoluble in dilute HCl (% on a dry mass basis) | not more than 0.12 |
| The acidity of extracted fat (as % oleic acid) | not more than 2.0 |

2.2.2 Muffin

Muffins are generally the small type of cake or quick bread which has a typical porous texture and high volume which gives a spongy texture (Martinez-carvera *et al.*, 2012). Muffins are made from high levels of sugar and fat. They are also referred to as "cupcakes" and are characterized by caramelized sweet flavour and attractive appearance. The word muffin is derived from the German word "*Muffen*" and spelt as moofin.

2.2.3 Role of bakery ingredients

The ingredients are classified into major and minor ingredients. The essential ingredients are flour, fat, sugar, water and chemical leavening agents (baking powder, sodium bicarbonate and ammonium bicarbonate). Minor ingredients include salt, flavour, milk powder emulsifiers and flavouring compounds (Mancebo *et al.*, 2015).

2.2.3.1 Flour

Usually, low protein & less damaged starch flour is used for biscuit making. Flour gives structure to the biscuit and holds an air bubble in it. It holds all the ingredients (fat, sugar, flavour & water) and ensures uniform mixing of ingredients. It retains air bubbles liberated by bicarbonate during baking (Goesaert *et al.*, 2005). Flour hydrates with water to form a protein network; hence it traps air bubbles in it. Soft wheat flour has finer granulation, less starch damage and low water absorption than hard wheat flour (Tanilli, 1976). Flour particle size is important, if the flour particle size is less, then the cookie spread will be more (Gaines, 1985). Most biscuits are made with flour with an average particle size of roughly 50 μm , of which less than 10% have a size more than 130 μm (Manley, 2011). When fine

particle size flour is used, it produces less development in terms of its characteristic attributes than with high-density biscuits (Mancebo *et al.*, 2015). The damaged starch and level of soluble starch in the flour are also important, as damaged starch absorbs more water and increases the viscosity and the viscosity of the aqueous phase was increased with more soluble starch (Gaines *et al.*, 1989). Generally, wheat flour used to make the biscuit contains 8-11% protein needed for proper sheeting and to form biscuit dough (Pareyt and Delcour, 2008). However, gluten in wheat flour causes celiac disease hence there is a need for alternative flour to make a biscuit.

2.2.3.2 Fat

The term "shortening" refers to a fat's capability to lubricate, soften, or shorten the structure of food ingredients so that they perform distinctively and give a food product desired textural feature (Ghotra *et al.*, 2002). The primary function of fat is to enhance flavour and mouthfeel by adding richness and tenderness (Lai & Lin, 2006). It also influences the bubble size and stability of the bubbles during creaming/battering and during baking (Kouhsari *et al.*, 2022). Fat minimizes the water absorption capacity of flour by surrounding the flour particle and breaking the continuity of the protein network and starch structure (Davidson, 2016). Thus, it prevents the formation of cohesive and extensible dough. Hence, contributes to the tenderizing effect of dough to form a soft & uniform textured biscuit (Mert & Demirkesen, 2016).

The secondary function of the fat is to entrap the air during the creaming stage and contributes to the leavening effect. This also influences the density of the product (Jacob & Leelavathi, 2006). Fats also limit the gelatinization process by reducing the quantity of water that the starch granules can absorb because of the complex between the lipid and amylose form during baking (Larsson, 1980). Pareyt *et al.* (2009) investigated the role of fat in sugar snap cookies that the weight and density of the dough decreased due to more air incorporation, the spread of a cookie increased due to the melting and mobility of fat, and cookie porosity was also increased with increase in fat level. The surface cracks were found when the fat level was decreased.

2.2.3.3 Sugar

Commonly sucrose is used for the preparation of biscuits and muffins. It contributes to the sweet taste & flavour of a product. Sugar influences the starch gelatinisation temperature, product water activity, crust colour by Maillard reaction and also the crispness of the product (Pareyt and Delcour, 2008). Sugar act as food to yeast thereby reducing the

fermentation time and producing carbon dioxide, involved in the aeration of the product. Sugar gives tenderizing action to a flour protein. The quality of the product is influenced by the type of sugar, granulation, and quantity of sugar used in biscuits. The particle size of the sugar influences the amount of sugar that goes into the solution and also the spread of the biscuit. Sugar competes for water and hence restricts the formation of gluten (Yamzaki & lord, 1971).

Sugar helps in the aeration of the dough during the cream stage (Chan, 2006). Sugar dissolves fully or partially in biscuit dough depending on the available water; if the undissolved sugar is left in the dough, then that will melt down during baking and help in spreading the biscuit and also it gives fragile crispness and crunchiness to a product (Arepally *et al.*, 2020). Sugar present in the baked goods regulates hydration and spreads out the particles of protein and starch, preventing a uniform mass from the formation (Maache-Rezzoug *et al.*, 1998).

A cookie's dough becomes less hard when there is too much sugar is added to it (Manohar & Rao, 1997). Typically, a higher sugar level causes a higher and longer oven rise, followed by a more noticeable collapse. This would imply that sugar affects both the amount of vertical expansion during baking and the timing of the chemical leavening activity (Chevallier *et al.*, 2000). When the sugar level increases the spread, cracks on the surface, porosity and break strength of the cookie increase, whereas the moisture of the cookie is reduced significantly (Pareyt *et al.*, 2009).

2.2.3.4 Salt

It adds flavour to the product, stops the activity of the yeast in the case of bread or cakes, extends shelf life through preservation action, and regulates water activity. Because yeast activity is dependent on the salt level, it indirectly affects a product's colour (Luchian & Canja, 2010). Being hygroscopic in nature, it helps in keeping moistness in a product. A poor crust colour results from insufficient salt levels, which promote yeast activity and reduce the amount of sugar available for caramelization (Belz *et al.*, 2012). Salt makes the gluten network stronger, which enhances the dough's ability to hold gas (Avramenko *et al.*, 2018). Since salt is hygroscopic, it helps to keep the product fresh and wet.

Ayed *et al.* (2021) reported that with the reduction of salt level, the aroma compounds like hexanal, nonanal and 2-pentyl-furan were also reduced. The moisture level decreased with decreasing salt levels. Salt does not add salt flavour to a product; rather it enhances the sweetness and masks the bitter, metallic and other off flavours (Miller and Hoseneey, 2008).

2.2.3.5 Baking powder

It acts as a leavening agent and contains sodium bicarbonate, acid & carrier-like starch. Baking powder should yield not less than 12% of available carbon dioxide (Powders, 1990). The bicarbonate contributes carbon dioxide and acid enhances/triggers carbon dioxide liberation. Aeration provides porous cell structure, fine grain & texture, increases the volume & decreases the density of a product (Canali *et al.*, 2020).

Canali *et al.* (2020) investigated the influence of different chemical leavening agents on sensory, textural and volatile compounds. He found that the ammonium bicarbonate secured the highest score due to the pronounced intensity of 2-methylpirazine, a chemical typically associated with nutty and roasted notes and low spread ratio & hardness in biscuits. Whereas the baking powder sensory score card was next to ammonium bicarbonate and the flavour compound produced were 1-hydroxy-2-propanone and 2-furanmethanol. The highest hardness and spread ratio were recorded in the same.

Ammonium bicarbonate is also known as 'Vol'. It's a leavening agent and does not require any additional acid to produce carbon dioxide (Pop, 2007). It does not produce gas at room temperature and decomposes under heat by producing carbon dioxide & ammonia. This ammonia is readily soluble in the water phase of dough and will remain in the product if a sufficient amount of is water available, which limits the use of vol (Gokmen *et al.*, 2008).

2.2.3.6 Emulsifier

Basically, an emulsifier is a substance when added lowers the interfacial tension between oil and water, thereby causing emulsification during homogenization. It assists in the air incorporation & stabilizes formed air bubbles in batter (Mahdi & Dawoud, 1986). The hydrophobic end of the emulsifier aligns with the fat & air phase and the hydrophilic end is located towards the water phase which stabilizes the air bubble (Kamel & Ponte, 1993). The most commonly used emulsifiers are Glycerol monostearate (GMS), lecithin and sodium stearoyl lactylate (SSL).

Emulsifiers can serve as anti-firming agents or crumb moisturizers that can complicate starch gelatinization, as well as dough strength that primarily interacts with gluten proteins (Sabanis & Tzia, 2011). In order to substitute the viscoelastic and gas-binding properties of gluten, hydrocolloids are frequently used to improve cake quality in wheat cake formulations (Eduardo *et al.*, 2014). Hydrocolloids also interact with the swelling, gelatinization, and gelling properties of the dough and the retrogradation of the starch (Arendt & Dal Bello, 2011).

2.2.3.7 Water

It is a minor ingredient used as a processing aid rather than an ingredient in biscuit preparation (Pareyt & Delcour, 2008). It helps in swelling of starch, hydration of gluten and ingredients to contact with the dough. It assists in the temperature control of dough and in dissolving dry ingredients such as salts, ammonium carbonate, sodium carbonate & ammonium citrate iron. To a certain extent causes aeration by forming steam (Stauffer, 2007). Water helps in the solubilization of salt, sugar, and leavening agents and dissipates the other ingredients in the dough therefore, it is necessary for dough formation (Manley, 2011). The amount of water needed to make cookie dough has an impact on how much gluten develops in the dough, how evenly the cookies spread during baking, how well the dough retains moisture, and how pleasant the finished products are to eat (Lai and Lin, 2006). Water controls the spread of the biscuit by controlling the viscosity of the dough, if the flour absorbs more water, then the available water will be less and viscosity will increase and resulting in less spread (Hoseney & Roger, 1994).

2.2.4 Dough/Batter rheology

The dough is made up of flour, sugar, fat and other main ingredients. Although the biscuit dough is cohesive in nature but lacks the extensibility and elasticity of the bread dough. Maximum strain is a measure of extensibility in biscuit dough, while per cent recovery is a measure of elasticity. The dough behaves viscoelastic below the yield strain and also at large deformation due to the weak protein network. During amplitude sweep, the stress increases vigorously with a small increase in the strain and the stress-strain curve follows the plateau-like region.

Knowledge of dough rheology is vital since it affects biscuit quality and process efficiency (Pareyt & Delcour, 2008). The use of mechanical and heat energy alters the rheological properties of dough. Furthermore, these qualities are controlled by numerous aspects like mixing methods, mixing equipment, mixing time, ingredient kind and quality, dough temperature, and water content (Manohar & Rao, 1999).

Frequency sweep tests were performed in the viscoelastic area acquired from amplitude sweep tests in order to obtain relevant information from the viscoelastic properties of different dough samples. The elastic or storage modulus (G') expresses the amount of energy stored in the material or recovered during each cycle of deformation. The viscous or loss modulus (G'') estimates the amount of energy lost per cycle of deformation due to viscous dissipation (Pedersen *et al.*, 2004). The biscuit dough shows the shear thinning

behaviour with an increasing shear rate. There are two terms i.e., flow behaviour index and consistency index. The flow behaviour index provides the information regarding shear thinning behaviour of the dough. The consistency index provides information regarding the ability of dough/ batter air retention. Abebe *et al.* (2015) studied the effect of tef grain flour addition on viscoelastic properties of dough. They found that the addition of tef flour reduced the viscoelastic property of the dough and increased the dough stickiness which affects the further machine operation. Mastromatteo *et al.* (2013) reported that the storage modulus and loss modulus decreased with the increase in the water content of the wheat dough. On the substitution of refined wheat flour with finger millet flour, the storage modulus and loss modulus increased but not more than the control which was made from refined flour (Kumar *et al.*, 2020).

One of the parameters that show the ratio of loss modulus (G'') and elastic modulus (G') is the loss factor ($\tan \delta$) (Jekle *et al.*, 2016). When $\tan \delta$ is less than one, G' is greater than G'' , indicating that elastic properties dominate over viscous properties in the dough (Zhang *et al.*, 2017). The tangent δ also talks about the organization of dough structure, if the dough has a lower tangent δ means the dough is highly structured. Also, the tangent δ increases with the mixing time and it depends on the protein-to-starch ratio (Mastromatteo *et al.*, 2013).

In the creep test, maximum strain represents the extensibility and per cent recovery represents the degree of recovery after relaxation (Wang & Sun, 2002). Pedersen *et al.*, (2004) reported that with the ageing of dough the maximum strain was decreased and the per cent recovery was increased, while the addition of protein increases the extensibility and did not affect the per cent recovery (Manohar & Rao, 1997).

2.2.5 Fat replacement

Fat is the primary component that gives cookies their suppleness, retaining quality, grainy, and texture, as well as their rich flavour (O'Brien *et al.*, 2003). The interaction of fat with other substances shapes the product's mouthfeel, texture, and overall lubricity perception (Stauffer, 1998). Additionally, fat affects the rheological characteristics of cookie dough (Jacob & Leelavathi, 2007).

A wide range of ingredients is being used as fat substitutes which provide distinct attributes to the product it was added to. Also, it is crucial to take into account how well these replacements work such that they can be compared with standard products. Sudha *et al.* (2007) reported that with the reduction of fat level, the biscuit dough becomes hard, more

springer and more cohesive. The physical property like the spread of biscuit reduced and the breaking strength of biscuit increased. Zoulias *et al.* (2002) investigated the texture of low-fat cookies using protein- or carbohydrate-based fat substitutes. They found that with the replacement of fat, cookies' hardness and brittleness increased.

Khalil (1998) studied the impact of emulsifiers and carbohydrate-based fat substitutes on the qualitative traits of low-fat cake. They found that with the replacement of fat, the specific gravity of the batter increased and cakes had the highest standing heights, specific volume, volumes, and compressibility than the full fat cake.

2.2.6 Egg substitute in cake/muffin

Nowadays, people prefer to eat vegan foods due to the health consequences of eggs (cholesterol, egg allergy & avian influenza). However, the prevailing consensus is that the egg is the necessary basic component for baked goods (Hosomi *et al.*, 1992). Due to their special foaming, solubility, emulsifying, and coagulating qualities, eggs are a crucial ingredient for achieving the ideal volume, texture, and colour in culinary products. In addition to their usefulness as a component, eggs also have flavour, colour, and nutritional value (Stadelman, 1999). Sometimes the cost of the egg goes beyond 50 % of the manufacturing cost of a product. So, the manufacturers are still finding cheaper ingredients to completely replace the egg. Lately, cohort studies have discovered egg substitutes such as whey proteins, soy flour, hydrocolloids, blood plasma, casein and starch in various bakery products. These egg replacers should give the same functional characteristics as an egg.

Whey proteins are the promising alternative which give the same functional properties to the products like foaming and foam stability (Morr *et al.*, 1973). The commercial eggless cakes are manufactured using whey proteins. Ratnayake *et al.* (2012) studied the effect of three different commercial egg substitutes (whey protein, fibre/gum, and soy/wheat gluten-based) on yellow cake. The density of the batter made with whey protein was comparable, baking loss was more in all egg substitutes compared to a cake made from eggs. The puncture test reveals that the cake made from whey had lower hardness than other substitutes. Jyotsna *et al.* (2007) incorporated whey protein concentrate at different levels in the eggless cake. They found that incorporation of up to 20 % whey protein concentrate (WPC) resulted in low specific volume, density and apparent viscosity. The volume of the cake was found to be more and the hardness was decreased. Lee *et al.* (2005) investigated the rheological properties of gluten-free cake in which the replacement of shortening resulted in a reduction of the viscosity and air incorporation levels and hardness got increased.

Saleh (2022) tried to develop to prepare a cake using novel egg substitute zucchini puree. In this study, it was found that the cake had acceptable characteristics which were mainly attributed to the gums present in the peel of zucchini. The batter density was higher for zucchini-based cake when compared to soy flour cake and the foam capacity and foam stability of zucchini-based cake were comparable with the cake made from soy flour. The viscosity of the zucchini batter was less compared to all other egg substitutes. Agrahar-Murugkar *et al.* (2018) studied the batter rheology; and noticed that the tangent δ and viscosity was higher for composite flour than the batter from refined wheat flour. Biaxauli *et al.* (2008) stated that with the addition of resistant starch to the batter, the apparent viscosity and consistency index was reduced. Further, the flow behaviour and linear viscoelastic properties showed a reduced complex structure with an increase in the level of resistant starch.

Aslan & Bilgiçli (2022) attempted to make a cake from lupin extract instead of soy extract and based on their investigation they concluded that the cake had a higher cake volume index and lower hardness. Hesarinejad *et al.* (2017) examined the effect of using *Chlorella vulgaris* as an alternative to egg whites in cakes and reported that the batter consistency and specific volume decreased and a significant increase in the weight loss and hardness was observed.

2.3 Ghee Residue: A Dairy By-product

Ghee residue is a by-product obtained during the preparation of ghee from cream or butter. Leftover material after ghee preparation is usually light to blackish brown in colour depending on the manufacturing process and temperature employed. Ghee residue consisted of residual/entrapped fat, highly denatured milk proteins, lactose, minerals and several other compounds formed due to lipid oxidation, Maillard reaction and membrane remnants. The average of fat and protein content in ghee residue varies in the range of 33.13 - 41.83 % and 30.91 - 31.69 %, respectively (Janghu *et al.*, 2014). The by-product obtained is usually considered waste with no apparent economic value but its richness in certain nutritive compounds including milk fat (30-70 %) makes it a potential substrate for lipase production (Sahasrabudhe *et al.*, 2012). However, on an industrial scale, ghee residue is utilized in the production of certain food products and as a flavour enhancer (Tamine, 2009). Traditionally, ghee residue has been widely used by mixing it with milk or skimmed milk powder, *khoa*, sugar and flavours for the preparation of sweets like chocolate, burfi, peda, *pinni*, toffees etc and in certain food preparations like soups, spreads etc (Verma and De, 1978 & Galhotra and

Wadhwa, 1993). In addition, its vast application in the food industry is limited mainly due to its poor oxidative stability. Ghee residue is highly prone to oxidation owing to higher levels of free unsaturated fatty acids which affect its flavour, colour, aroma, nutritional value, rancidity etc (Pawar *et al.*, 2014). Hence, utilization of ghee residue immediately after production is important as it may lead to the rapid development of rancidity thereby reducing product shelf-life and ultimately consumer acceptability (Nerin *et al.*, 2008).

The Physico-chemical characteristics of ghee residue obtained using different methods of manufacturing are depicted in Table 2.4. The concentration of different components in the same varies from method to method when the raw materials used (Fat and milk serum) are heated at an average temperature of 120 °C/ 10-20 minutes. Another important aspect of ghee residue with respect to ghee is that it contains about 10, 11 and 132-times higher levels of carbonyls, free fatty acids and lactones, respectively (Table 2.5) (Verma and Raju, 2008) and hence it can be easily admixture with a number of products such as candies, *laddu*, burfi, sweets etc. Ghee residue contains higher levels of phospholipids and thus higher levels of natural antioxidant properties (Sojan *et al.*, 2019).

Table 2.4: Physico-chemical attributes of ghee residue produced by different methods

| Source of Ghee residue | Average fat % (starting material) | Chemical composition of ghee residue | | | | | | Yield (Kg/100Kg) |
|--------------------------------------|-----------------------------------|--------------------------------------|------|---------|---------|-----|----------------|------------------|
| | | Moisture | Fat | Protein | Lactose | Ash | Phospho lipids | |
| Desi butter from buffalo milk | 77 | 13.4 | 33.4 | 32.8 | 15.4 | 5.2 | 4.95 | 1.6 |
| Creamery Butter (Unsalted) | 85 | 5.7 | 65 | 25.5 | traces | 3.8 | 17.39 | 1.2 |
| Sweet Cream | 67 | 4.1 | 63.2 | 18 | 12.3 | 2.4 | 1.57 | 7.7 |
| Sour Cream | 67 | 8 | 38.8 | 41.6 | 7.3 | 4.3 | - | 5.1 |
| Washed Sweet Cream | 71 | 1.7 | 80.8 | 16.2 | trace | 1.3 | - | 3.5 |

Source: Aneja *et al.*, 2002

The comparison between ghee and ghee residue is shown in Table 2.6. Further, when the fat extract from the ghee residue was subjected to determine its fatty acid profile showed that among saturated fatty, it contains higher levels of palmitic acid (~38.88 %) and among

unsaturated fatty acids i.e., oleic acid, Linoleic, Linolenic, eicosatetraenoic and docosahexaenoic acid is 25 %, 2.02 %, 0.79%, 0.36 % and 0.25 %, respectively (Ranjan *et al.*, 2020). The amino acid profile revealed that ghee residue is rich in glutamic acid (~5.26 %) while cysteine was the lowest (~0.35 %), the detailed fatty acid profile, amino acid profile and protein quality of ghee residue are shown in Table 2.5. The ghee residue was analysed for its PER, BV and NPN value and the results obtained showed that it has an average of ~0.75, ~65.07 % and ~40.77, respectively (Grewal, 1979).

Ghee residue is the moist, partially strained; burnt & light to dark brown sediment that is obtained on the cloth strainer/nylon filter after ghee is filtered. It is largely made of the solid-not-fat present in cream or butter appearing in the form of small particles during the manufacture of ghee.

Table 2.5: Fatty acid and amino acid profile of ghee residue

| Fatty acid | Average % | Amino acid | Average % |
|-----------------------------------|------------------|-------------------|------------------|
| Myristic acid | 13.38 | Alanine | 0.87 |
| Palmitic acid | 38.88 | Arginine | 1.95 |
| Stearic acid | 12.72 | Aspartic acid | 2.48 |
| Arachidic acid | 0.25 | Cysteine | 0.35 |
| Behenic acid | 0.32 | Glutamic acid | 0.40 |
| Palmitoleic acid | 2.20 | Glycine | 0.52 |
| Oleic acid | 25.15 | Histidine | 0.99 |
| Linoleic acid | 2.02 | Isoleucine* | 0.50 |
| Linolenic acid | 0.79 | Leucine* | 2.85 |
| Eicosapentaenoic acid | 0.36 | Lysine* | 0.58 |
| Docosahexaenoic acid | 0.25 | Methionine* | 0.45 |
| Protein quality | | Phenylalanine* | 1.02 |
| Protein Efficiency Ratio (PER) | 0.75 | Proline | 2.36 |
| Biological value (BV) (%) | 65.07 | Serine | 0.54 |
| Net protein utilization (NPU) (%) | 40.77 | Threonine* | 1.44 |
| Digestibility co-efficient | 62.65 | Valine* | 1.74 |

(Note: Saturated Fatty Acid (%) (SFA): 65.5 Unsaturated Fatty Acid (%) (USFA): 34.5; SFA: USFA: 1.9:1; * indicates Essential Fatty Acids) (Ramesh *et al.*, 2018 & Selvamani *et al.*, 2017)

2.3.1 Physico-chemical & Functional properties of ghee residue

Ghee residue is moist, glossy exterior (covered with fat) and smooth to granular texture. The average particle diameter of ghee residue is 104.79 μm and the average density of ghee residue particles is 1.14 g/cm^3 (Aneja *et al.*, 2002). As mentioned earlier the colour of

ghee residue is light to dark brown depending upon the starting material and intensity of heating during ghee manufacturing.

Table 2.6: Comparison between cow milk ghee, buffalo milk ghee and ghee residue

| Parameter | Ghee residue | Buffalo ghee | Cow ghee |
|-------------------------------------|--------------|--------------|----------|
| Iodine value | 43.4 | 29.4 | 33.70 |
| Reichert meissel value (RM) | 24.4 | 32.3 | 26.7 |
| Polenske value (PV) | 1.3 | 1.41 | 1.76 |
| Flavour compounds | | | |
| Flavour compounds | Ghee residue | Ghee | |
| Carbonyls ($\mu\text{m/g}$) | 43.4 | 4.3 | |
| Free fatty acid ($\mu\text{m/g}$) | 627.5 | 53.6 | |
| Lactones ($\mu\text{m/g}$) | 3992.9 | 30.3 | |

Source: (Aneja *et al.*, 2002)

Table 2.7: Nutrient composition of ghee residue per 100 gm

| Nutrients | Nutritive value |
|---------------------------|-----------------|
| Energy kcal/100g | 893.50 |
| Fat g/100g (on db) | 50.25 |
| Protein g/100g (on db) | 25.07 |
| Moisture% | 13.28 |
| Ash g/100g (on db) | 13.28 |
| Calcium mg/100g | 0.91 |
| Total carbohydrate g/100g | Traces |

2.3.1.1 Chemical composition of ghee residue

The moisture, ash & lactose content of ghee residue from the desi butter method is more, protein is higher in the sour cream method & fat level is more from the creamery butter method.

Protein: Heat treatment denatures the protein and decreases the soluble nitrogen with increasing heating time. β -lactoglobulin is the main source of sulfhydryl protein from whey, these proteins are mostly retained in the ghee residue due to its polar nature. The total reducing capacity expressed as free sulfhydryl content ($\mu\text{m/g}$) of creamery butter ghee residue is 2.90 and mg of cysteine hydrochloride/g is 26.0 which is found to be higher than in ghee (0.02 and 0.075, respectively). The details of the different amino acids present in ghee residue are shown in Table 2.5.

Milk sugar: Percentage of different sugars in ghee-residue prepared at 120°C is lactose (5.50-17.88), galactose (15-20), glucose (5-10) & others. These levels vary depending upon the method of preparation. However, with increasing heat treatment while preparing ghee,

further degradation of lactose is observed which results in the formation of glucose and galactose (Santha and Narayanan, 1978). This can be a better option for lactose intolerant people looking for low lactose food products and also this by-product i.e., GR can become an economical source for milk solids for low-lactose product formulations.

Fat in ghee residue: The ghee residue has a higher iodine value and lower Reichert Meisel (RM) and polenske value (PV) when compared to ghee from buffalo & cow. This is because ghee residue contains lower levels of short and medium chain and saturated fatty acid (4-12%). However, the higher PUFA content makes it susceptible to oxidation, thus limiting its shelf-life. Recovery of phospholipids a major nutraceutical in ghee residue was maximum from the creamery-butter method (17.39%) than other methods and in ghee (0.004-0.08%). Most of the phospholipids remain in ghee residue due to their polar character. The different types of fatty acids quantified in ghee residue are shown in Table 2.5.

Minerals: Ghee residue is found to be rich in both micro and macro minerals which have better bioavailability. The GR contains calcium, magnesium and phosphorus in the range of 0.54-0.62%, 0.57-0.61% and 0.52-0.64, respectively. The trace elements like iron, copper and manganese were found to be around 401.07-427.15 ppm, 5.84-7.75 ppm, and 318.50-392.48 ppm, respectively (Selvamani *et al.*, 2017; Gueguen & Pointillart, 2000)

2.3.1.2 Flavour properties

Ghee residue is a rich source of natural flavour compounds like carbonyls, lactones and free fatty acids and their concentration is 10,132 & 11 times higher than ghee, respectively (Verma and Raju, 2008). The average values for carbonyls and δ -lactones were 43.65 $\mu\text{mol/g}$ and 237.32-533.62 $\mu\text{g/g}$ of ghee residue, whereas the FFA content was 627.48 $\mu\text{mol/g}$ (Galhotra and Wadhwa, 1993). The different flavour compounds present in ghee and ghee residues are mainly due to the heating process applied during ghee making. The heating process assists in the elimination of off-flavours. The carbonyls in ghee residue are formed majorly through three pathways i.e., thermal degradation of lactose and glycerides, as microbial metabolites and oxidation of fat (Kumbhare *et al.*, 2021).

2.3.1.3 Antioxidant properties

Ghee residue is a good source of phospholipids (lecithin, cephalin, sphingomyelin and cerebroside) and nitrogenous compounds. Most of these compounds are responsible for the antioxidant property. Among phospholipids, cephalin showed greater antioxidant activity in

comparison to other forms. The antioxidant property of ghee residue depends on the manufacturing process and the temperature of clarification. An increase in clarification temperature decreased the antioxidant efficacy of the residue. Non-lipid constituents like proline, lysine, tryptophan, cysteine and free sulfhydryl compounds also contribute to the antioxidant properties (Nooshkam *et al.*, 2019; Meena *et al.*, 2021).

2.3.2 Application

Ghee residue is used in different food/sweets preparation at a laboratory or pilot scale, however; most of the above-mentioned products are not produced on a commercial scale and do not belong to popular variants. *Pinni* is the only product based on ghee residue which is a commercialized on large scale. However, ghee residue has been utilized for non-food applications as well. Ghee residue is used as a solid substrate for the production of lipase enzyme using *Bacillus subtilis* and higher lipase production was observed in solid state fermentation (35.93 U/mg) than the submerged liquid fermentation (28.63 U/mg) (Sahasrabudheet *et al.*, 2012).

Dua *et al.* (2018) prepared a burfi using ghee residue by replacing *khoa* with ghee residue (up to 50 %) and the product with 60 % *khoa* and 40 % ghee residue was optimized based on sensory evaluation. The sensory scores for flavour, body and texture were higher compared to the *khoa* based burfi. Reddy and Khan (1978) prepared chocolate using ghee residue, sugar, cocoa solids, and skim milk powder and evaluated it for consumer acceptance. The ghee residue-based chocolate was compared with the different brands of market chocolate. The developed chocolate was highly acceptable than the market chocolate. Wadhwa (1997) prepared candy using ghee residue, sugar & coconut powder. Also, Janghu *et al.* (2014) determined the storage stability of ghee-residue based products and observed that the free fatty acid content enhanced during the storage period when packed in polyethene pouches and glass containers.

Thabdi is a traditional sweet of Gujarat state, has characteristics of brown colour with a caramelized, nutty and cooked flavour with a loose granular texture and leaky fat. Hirpara *et al.* (2020) prepared *Thabdi* sweet by utilizing ghee residue by replacing *khoa* at levels varying from 2-10 % to adjust the fat to solid not fat (SNF) ratio of 0.53. The 6 % ghee residue added to *Thabdi* had a maximum overall acceptability score. By substitution of *khoa* with the ghee residue, the protein, fat, and ash contents were increased.

Pinni is a milk cereal-based sweet, has a typical dark brown colour with a granular texture and is a popular sweet in the states of Haryana, Delhi and Punjab. *Pinni* is produced

by roasting refined wheat flour followed by the addition of dry fruits and ghee residue, followed by the addition of sugar along with tragacanth gum (Pranav and Meena, 2018). The developed product was appreciated by the panellist.

Rajan *et al.* (2020) attempted to prepare cake and muffin by replacing refined wheat flour with ghee residue (10-40 %). The cake & muffins made from 60 % refined wheat flour and 40 % ghee residue had highest overall acceptability. The body & texture, flavour and taste were improved compared to the control (without ghee residue) sample. The product was nutritionally better, especially in calcium content. Sojan *et al.* (2019) developed cookies and biscuits by replacing the bakery fat with ghee residue. It was noticed that up to 10 % shortening can be replaced with GR and that reduced the cost of production by ~16.6 %.

Table 2.7: Application of ghee residue in food products

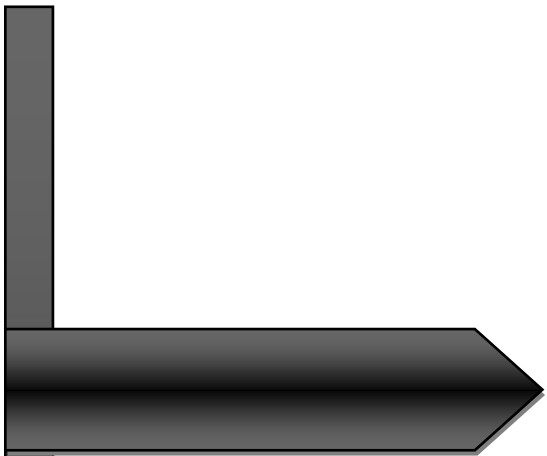
| Food product | Reference |
|-------------------------------------|------------------------------------|
| Cakes & biscuits | Subbulakshmi <i>et al.</i> , 1990 |
| Candy, chocolate & burfi type sweet | Janghu <i>et al.</i> , 2014 |
| Edible paste | Prahlad, 1954 |
| Chocolate burfi | Reddy & Khan (1978), Wadhwa (1997) |
| Burfi supplemented with corn flour | Dua <i>et al.</i> , 2018 |
| Flavour enhancer | Tamine (2009) |
| Confectionery food product | Prahlad, 1954 |
| Pinni | Pranav & Meena, 2018 |
| Thabdi milk sweet | Hirpara <i>et al.</i> , 2020 |

2.3.3 Technological development in Ghee-residue utilization

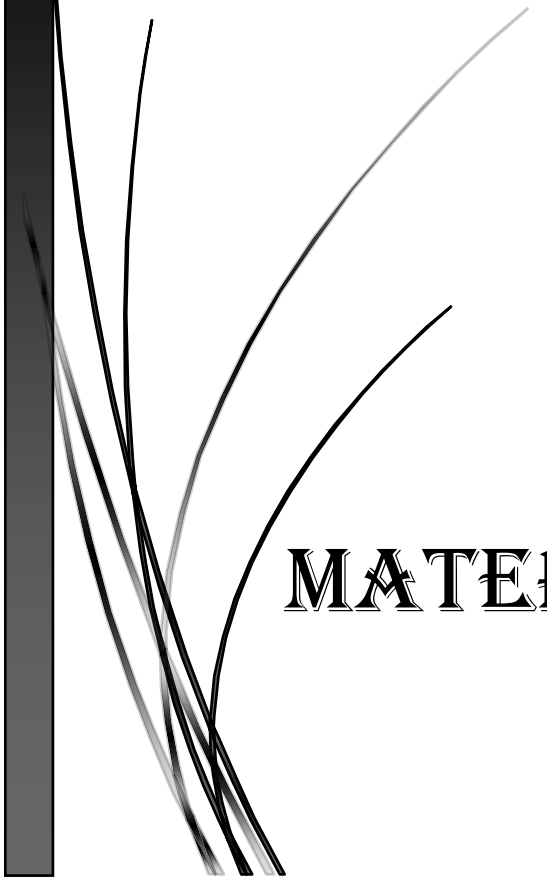
Ghee residue powder is made by mixing with water or skim milk or buttermilk, homogenizing the mix and spray drying at standardized temperatures. On average, it contains 96.44-98.22% total solids, 24-43% fat and 32.5-42% protein (Gill, 2021). In another investigation Munirathnamma *et al.* (2017) attempted to recover the proteins from ghee residue by application of different methods like acid precipitation, boiling in water, using 50% alcohol and also by ultrafiltration. All of these methods except ultrafiltration failed to extract the proteins from GR. Ultrafiltration was able to recover the proteins (up to 97 %) with a purity of 70 %. Thalor (2021) manufactured the ghee residue powder by admixing fresh ghee residue with buffalo skim milk. The admixture was tested for heat coagulation and due to the low pH of ghee residue, coagulation occurred, hence to overcome such problems

stabilizing salts (tri-sodium citrate and sodium tri-polyphosphate) were added. Then the mixture was heated indirectly to 60⁰C and homogenized in a double-stage homogenizer (2500 Psi & 500 Psi). Then the homogenized solution was spray dried and the powder was packed in a metallized polyethylene pouch.

In the view of health benefits of ghee residue and ghee residue powder, the shortening could be replaced to make the biscuits and muffins. Both GR and GRP are nutrient-rich, exhibits unique functional properties and hence can be used as an ingredient in preparation of bakery products without adversely affecting their quality characteristics.



CHAPTER 3



MATERIALS AND METHODS

3.0 Materials & Methods

This chapter deals with the experimental details including technological, analytical and statistical aspects related to the manufacturing of biscuits & muffins from ghee residue & ghee residue powder. The make and specifications of the various equipment used in this investigation are also included.

3.1 MATERIALS

3.1.1 Pearl millet flour

Good quality pearl millet flour was procured from B.D Super store market, Karnal, Haryana

3.1.2 Refined Wheat Flour (RWF)

Good quality refined wheat flour/ maida (Brand name: Rajadhani™) was procured from B.D Super store market, Karnal, Haryana

3.1.3 Hydrogenated vegetable fat

Good quality hydrogenated vegetable fat (Brand name: Marvo pride™) was procured from the local market. It was stored and placed in a cool and dry place.

3.1.4 Ghee-residue

Fresh ghee-residue sample was procured from the Model Dairy Plant of the ICAR-National Dairy Research Institute, Karnal (Haryana). The ghee-residue was obtained after the recovery of milk fat by steam stripping method and the image was shown in figure 3.1 (A).



Fig 3.1: (A) Ghee Residue (B) Ghee residue powder

3.1.5 Skim milk powder

Skim milk powder was obtained from Modern Dairies Limited, Karnal, Haryana.

3.1.6 Ghee residue powder (GRP)

GRP was manufactured from fresh ghee residue obtained from the Model Dairy Plant of the ICAR-National Dairy Research Institute, Karnal (Haryana). It was prepared according to the method mentioned by Thalor (2021) and the GRP image is shown in the figure 3.1 (B).

3.1.7 Sugar

Commercially available good quality crystal sucrose was purchased from the B.D Super store market, Karnal, Haryana

3.1.8 Salt

Commercially available good quality crystal iodised salt (TATA®) was obtained from the local market of Karnal.

3.1.9 Whey Protein Concentrate

Whey protein concentrate (WPC 70) with specifications as detailed in Table 3.1, was procured from Modern Dairies Private Limited, Karnal, Haryana.

Table 3.1: Specification of whey protein concentrate

| Parameter | Value |
|--------------------------------|--------------|
| Colour | Creamy white |
| Taste | Normal |
| Flavour | Clean |
| Moisture % | 3.84 |
| pH | 6.75 |
| Milk fat % | 5.80 |
| Ash % | 3.86 |
| Scorched particles (ADPI Disc) | A |
| Protein % | 70.84 |
| SPC/g | 3200 |
| Coliform/ 0.1 g | Negative |
| <i>E. coli</i> / g | Absent |
| Salmonella /25 g | Absent |
| Staphylococcus aureus/ g | Absent |
| Yeast and mould/ g | 20 |

3.1.10 Flavour

The vanilla flavour was procured from International Flavours and Fragrance India Pvt. Ltd., Chennai.

3.1.11 Baking powder

Double-acting *baking powder* and *Cake gel* were procured from the local market of Karnal. It was placed in a cool and dry place. Cake gel was a mixture of emulsifiers: INS 471 (Mono and diglycerides of fatty acids) and INS 477 (Propylene glycol esters of fatty acids) and humectants: INS 1520 (propylene glycol) and INS 422 (Propylene glycol esters of fatty acids).

3.2 Chemicals and reagents

All the chemicals used for the preparation of different reagents and for carrying out chemical analysis were of Analytical Grade (AR) and were procured from standard suppliers. The reagents required for analysis were prepared fresh adopting standard procedures.

3.3 Glassware

Glassware used was of Tarson and Borosil brand and wash thoroughly cleaned by soap solution, dried & used.

3.4 EQUIPMENT AND INSTRUMENTS

Following equipment and instruments were used during the preparation of ghee residue powder, biscuit & muffin and also in the analysis of ghee-residue & ghee residue powder, flour, biscuit and muffins.

3.4.1 Stainless steel (SS) sieve

Fresh ghee-residue was passed through SS sieve (mesh size- 1.45mm) to eliminate the burnt particles and other foreign materials. This also served the purpose of pulverization.

3.4.2 Double jacketed steam kettle

A double jacketed steam kettle (model & make), located at the BPD unit was used to indirectly heat treat the mixture of skim milk and ghee-residue in batch mode.

3.4.3 Homogenizer

A double stage homogenizer (model: C3534; make: Crepaco, Chicago, IL, USA) located in experimental dairy of the institute was used to homogenize suspension comprises of ghee residue and skim milk.

3.4.4 Spray dryer

Single stage spray dryer (Jektrons Engineers Pvt. Ltd., Bhosari, Maharashtra) located in the experimental dairy of the institute was used to manufacture powder sample. This drier was equipped with a rotary atomizer (RPM: 18,000-22,000), having a feed rate of 110 kg/h and water evaporation capacity of 60 kg /h.

3.4.5 Heat sealing machine

Manufactured biscuit products were subsequently filled in metalized polyester-LDPE laminate pouches and sealed using a heat-sealing machine (model: Sepack 300 HB; make: Teknik Industrial Trades, Ambala, India).

3.4.6 Hot air oven

A hot air oven (model: ABCD India; make: The Laboratory Glassware Co., Ambala Cantt., India) was used for the estimation of total solid (ghee residue) and moisture (biscuit, muffin & flour) for the gravimetric analysis of other materials.

3.4.7 Muffle furnace

After charring, the ash content of different samples (ghee-residue, ghee residue powder, bajra & wheat flour, biscuit and muffin) was determined using a muffle furnace (Modern Industries Corporation, Bombay, India).

3.4.8 Protein digestion and distillation equipment

A protein digester (Model: KES 06L VA DLS KEL PLUS; make: Pelican equipment, Chennai, India) was used to digest protein present in different samples (ghee-residue, pearl millet flour & wheat flour, biscuit and muffin). A distillation unit (Model: KELPLUS SUPRA – LX VA; Make: Pelican equipment, Chennai, India) was used to capture ammonia produced during the boiling of digested material.

3.4.9 Double distillation unit

A double distillation unit (make: Bhanu Scientific Instruments, Mumbai, India) was used to produce double distilled water.

3.4.10 Tap density tester

Loose bulk density (LBD), packed bulk density (PBD) also known as tapped bulk density, compressibility index (CI) and Hausner ratio (HR) of ghee residue powder were determined using a tap density tester (model: ETD 1020; make: ELECTROLAB India PVT. LTD., Mumbai, India).

3.4.11 Colour meter

(Hunter lab, Reston, Virginia, USA) colorimeter equipped with dual beam xenon flash lamp and universal software was used for measurement of the colour of the product in terms of L*, a* and b* colour coordinates

3.4.12 Hobart Mixture

Hobart planetary mixer (bowl capacity, 5L) fitted with the mixing hook (flat beater) manufactured by Hobart Corporation, U.S.A. was used for creaming and mixing of raw materials.

3.4.13 Water activity meter

The water activity meter of Aqua lab (Model Series 3 TE) supplied by M/s Decagon Devices, WA, USA was used for the determination of the water activity of dough, biscuits & muffins.

3.4.14 Texture analyzer

Textural attributes were evaluated by using TA-HD plus texture analyser (Stable Micro System, USA). fitted with a 50 Kg load cell.

3.4.15 Rheometer

A Rheometer (model; MCR52; make: Anton Paar, Germany) equipped with measurement probes (PP50) was used to evaluate the amplitude sweep, frequency sweep, temperature sweep, flow sweep and creep test of dough and batter.

3.4.16 Convection Oven

A convection oven was used for baking muffins batter. It was procured from HCS Enterprises. Specification of convection oven given by supplier as stated below:

| | |
|------------------------|--------------------|
| Model number | HCS-Minj |
| Electrical Requirement | 2 hp |
| Type of control panel | Electro-mechanical |

3.5 METHODOLOGIES

3.5.1 Ghee residue powder preparation

GRP was manufactured according to the method mentioned by Thalor (2021). Fresh ghee residue was procured from the Model Dairy Plant, Karnal. The ghee residue preliminary analysis was carried out for its composition and suitability for spray drying. A Small portion

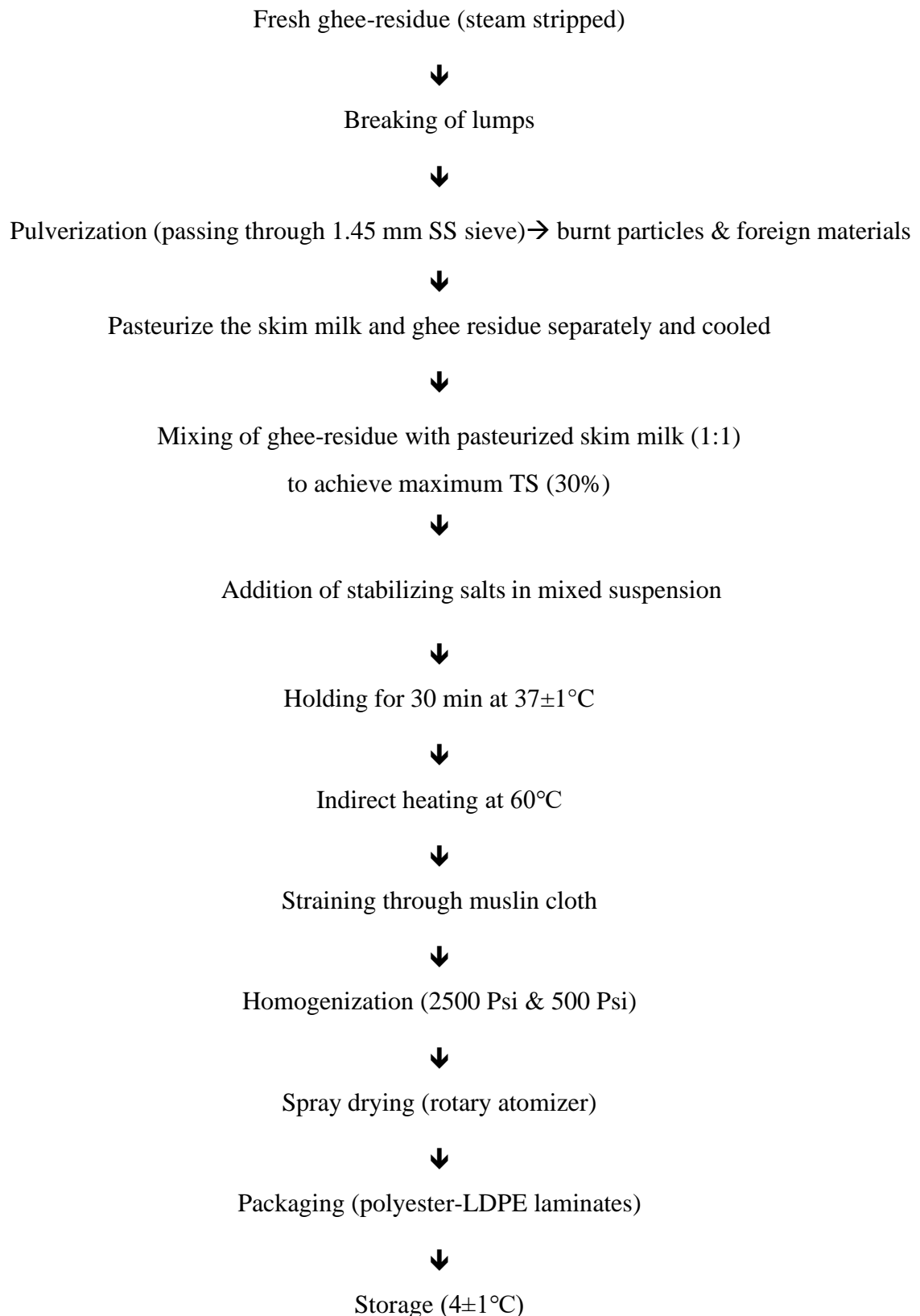


Figure 3.2: Flow chart for the production of ghee residue powder

of ghee residue was mixed with the skim milk in the ratio of 1:1 and was checked for its heat stability. The admixture of skim milk and ghee residue was not heat stable hence stabilizing salts (Sodium tri polyphosphate and Trisodium citrate) were added @ 0.6% and 1.0%. From the trials, it was noted that the suspension containing 0.6% stabilizing salt also forms the visible flake formation/curdling on heat treatment hence the 1.0% stabilizing salts were added to the suspension and it was found that the suspension is heat stable.

Fresh ghee residue was first manually pulverized by passing them through a SS sieve (mesh size- 1.45mm) to break the lumps and remove the burned particles & other foreign materials. Then it was pasteurized and cooled to 30°C. On the other hand, skim milk was pasteurized and cooled to 30°C separately. Now the stabilizing salt is added a @ 1.0% to skim milk, thereafter ghee residue was added into it (1:1) to form a suspension. This suspension was heated to 60°C indirectly by immersing the stainless steel can in hot water. and subjected to the homogenization process using a double-stage pilot scale homogenizer (2500 Psi & 500 Psi). Then the homogenized solution was spray dried using a single-stage spray drier equipped with the rotary atomizer. To avoid the lumping of powders during storage, the manufactured powder was packed under hygienic conditions in the pre-sterilized polyester-LDPE laminates pouches and stored at 4±1°C in the refrigerator till further use.

3.5.2 Preparation of biscuits

3.5.2.1 Mixing of dry ingredients

Calculated quantities of WPC, baking powder and common table salt were scaled and they were added to scaled flour, then sieved together and transferred to a dry container.

Table 3.2: Ingredients used for biscuit preparation

| Ingredients | Percentage |
|---|----------------|
| Refined flour: Pearl millet flour (50:50) | 100 |
| Bakery fat | 35 |
| Refined oil | 5 |
| Ground sugar | 40 |
| Skim milk powder | 4 |
| salt | 1 |
| Baking powder | 1 |
| Ammonium Bicarbonate | 0.6 |
| Sodium Bicarbonate | 0.4 |
| Ammonium iron citrate | 2 mg/ kg flour |

3.5.2.2 Addition of iron salt

Iron salt was added to the dried ingredients at the rate of 2 mg/ 1000 g of flour and mixed well.

3.5.2.3 Creaming of fat and sugar

Accurately weighed quantities of fat and sugar were taken and rubbed together to a creaming consistency.

3.5.2.4 Addition of water to the cream

Ammonium bicarbonate was mixed with the water and added to the cream slowly with continuous mixing in a Hobart mixer.

3.5.2.5 Addition of ghee residue/ghee residue powder and kneading

Ghee residue and ghee residue powder were added at different levels (10 %,15 %, 20 % & 25%) by replacing bakery fat. Previously mixed and weighed ingredients and ghee residue/ghee residue powder were added to the cream slowly with continuous kneading at slow speed in the Hobart mixer until the dough attains smooth homogenous mass.

3.5.2.6 Rolling and cutting

The dough was rolled out into a thin sheet of 2–3 mm thickness and 4 mm diameter by means of a wooden rolling pin and the sheets were then cut into the desired shape using a biscuit cutter mould.

3.5.2.7 Baking

The cut pieces were baked at 175°C for 13±3 minutes in the oven.

3.5.2.8 Cooling and packaging

The baked biscuits were cooled to room temperature and packed.

3.5.3 Effect of dough resting time and different levels of baking powder on ghee residue added biscuit

To see the interaction effect of dough resting time and baking powder level on the Physico-chemical characteristics of the biscuit, the optimal amount of ghee residue/ghee residual powder added biscuit was chosen.

Table 3.3: Composition of ghee residue substituted dough and biscuits

| Code for dough | Code for biscuit | Composition details |
|----------------|------------------|--|
| WBD50 | Control | Refined Wheat flour (50%) + Pearl millet flour (50%) + Shortening (100%) |
| WBDGR10 | 10 % GR | RWF (50%) + PMF (50%) + Ghee Residue (10%) + Shortening (90%) |
| WBDGR15 | 15% GR | RWF (50%) + PMF (50%) + GR (15%) + Shortening (85%) |
| WBDGR20 | 20% GR | RWF (50%) + PMF (50%) + GR (20%) + Shortening (80%) |
| WBDGR25 | 25% GR | RWF (50%) + PMF (50%) + GR (25%) + Shortening (75%) |

Table 3.4: Composition of ghee residue powder substituted dough and biscuits

| Code for dough | Code for biscuit | Composition details |
|----------------|------------------|--|
| GRP10 | 10 % GRP | RWF (50%) + PMF (50%) + Ghee Residue Powder (10%) + Shortening (90%) |
| GRP15 | 15% GRP | RWF (50%) + PMF (50%) + GRP (15%) + Shortening (85%) |
| GRP20 | 20% GRP | RWF (50%) + PMF (50%) + GRP (20%) + Shortening (80%) |
| GRP25 | 25% GRP | RWF (50%) + PMF (50%) + GRP (25%) + Shortening (75%) |

3.5.4 Preparation of muffins

3.5.4.1 Sieving

All the dry ingredients (refined wheat flour, baking powder, WPC- 70) were sieved together using a 12-micron mesh size sieve for proper mixing.

3.5.4.2 Creaming

Creaming of the shortening was done at medium speed (418 rpm) for 5 minutes. The sugar and shortening were creamed together at the same speed and time. The sugar crystals aid the incorporation of air bubbles into the mixture, and the density is reduced as the mixing continues, which can be seen by the progressive ‘whitening’ of the mixture. The cake gel was added to the mixture and it was creamed for 2 minutes to improve the quality of the batter and final product.

Table 3.5: Ingredients used for muffin preparation

| Ingredients | Percentage |
|------------------|------------|
| Flour | 24 |
| Bakery fat | 16 |
| Ground sugar | 24 |
| WPC | 6 |
| Skim milk powder | 2 |
| Baking powder | 1.2 |
| Cake gel | 0.5 |
| Vanilla | 0.3 |

(Singh, 2012)

The WPC was replaced with Ghee residue powder by 25 %, 50 %, 75 % & 100 %.

Table 3.6: The code given for the composition of different treatments

| Treatments code | Composition details |
|-----------------|---|
| W | Refined Wheat flour (100 %) + 100 % WPC |
| WB | Refined Wheat flour (50 %) + Pearl millet flour (50 %) +100 % WPC |
| WB25 | RWF (50 %) + PMF (50 %) + 25 % GRP + 75 % WPC |
| WB50 | RWF (50 %) + PMF (50 %) + 50 % GRP + 50 % WPC |
| WB75 | RWF (50 %) + PMF (50 %) + 75 % GRP + 25 % WPC |
| WB100 | RWF (50 %) + PMF (50 %) + 100 % GRP + 0 % WPC |

3.5.4.3 Mixing

In this step WPC and ghee residue powder were added to the fat–sugar mixture in four to six individual portions with in-between blending after the addition of each portion so that uniform dispersion can be obtained without deaeration of the mixture. It was done at slow speed (218 rpm) for 3 minutes. Then flour and baking powder was added to the mixture

and blended continuously for 3 minutes at medium speed. Finally, the liquids e.g., vanilla and water were added and blended at low speed (218 rpm).

3.5.4.4 Moulding and Baking

The batter was filled into the muffin moulds which were pre-cover with the muffin cover and was baked at 180°C for 18 minutes in the conventional oven.

3.6 ANALYTICAL METHODS

All the chemicals used in the present study were of analytical grade (AR) and procured from reputed suppliers. The reagents required for analysis were freshly prepared from chemicals by adopting standard procedures/protocols. The procedures were standardized and reagents were stored under desired conditions wherever required.

3.6.1 Physical/Physico-chemical analysis

3.6.1.1 Moisture

Moisture content was determined in triplicate as per AACC (1999) procedure. Five-gram sample was taken in a clean dried aluminium dish and weighed. The content was dried in the oven at 105 ± 3°C for four hours till a constant weight was obtained and cooled in a desiccator. After cooling, the loss in weight was taken as moisture content and expressed in terms of percentage

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

Where,

W₁- Weight of empty aluminum dish

W₂- Weight of aluminum dish with sample before drying

W₃- Weight of aluminum dish with sample after drying

3.6.1.2 Total Solids (TS)

The TS contents of ghee residue was determined by adopting the gravimetric method of BIS (2001a). In this method, clean and washed aluminium dishes were kept in the hot air oven at 102±2 °C for 1 h. The dishes were cooled in a desiccator for 30 to 40 min. Thereafter, 5 g of samples were accurately weighed in pre-weighed dishes. The dishes were then placed in the hot air oven, maintained at 102±2°C for 3 h. The dishes were removed, placed in the desiccator, cooled and weighed. The dishes were placed back in the oven for another 1 h, removed, cooled in a desiccator, and weighed again. The process of heating, cooling and weighing was repeated every 1 h till consecutive readings with a difference of

not more than 0.5 mg was obtained. From the amount of dried residue left in the dishes after evaporation of moisture, TS content was calculated using the following formula:

$$\text{Total solids (TS, \%)} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

Where,

W₁=Weight of empty dish taken for test

W₂= Weight of dish with sample

W₃=Weight of content in dish after drying

3.6.1.3 Fat

3.6.1.3.1 Ghee Residue

The fat content of ghee-residues, was estimated by the method reported in BIS (BIS 1986: 11721) for casein/caseinate. One gram of this sample was weighed (nearest to 0.1 mg) into the fat extraction Mojonnier flask and 10 mL of hydrochloric acid [prepared by diluting 675 mL of concentrated hydrochloric acid (density 1.18 g/ mL) to 1000 mL of distilled water] was added to it. The mixture was gently heated by placing it in a boiling water bath for 1 h and then cooled in running water. Ten mL ethanol (>94%, w/v) was added to the mixture, and mixed gently between the two bulbs of the flask by backward and forward motion. Twenty-five mL diethyl ether (Sp.gr.0.72) was then added to it and the tube was tightly closed with bark cork or glass stopper followed by vigorously shaking for 1 min. Twenty-five mL of light petroleum ether (boiling point 40-60°C) was then added to it and mixed vigorously for 1 min. The tube was allowed to stand for not less than 30 min. The ether layer was carefully decanted into a previously dried, cooled and weighed 100 mL conical flask. The extraction and decantation were repeated twice by using 15 mL each of diethyl ether and petroleum ether. The solvent was evaporated on hot plate and the residual fat was dried in the hot air oven at 102± 2°C for 1 h. The flasks were cooled in a desiccator. Drying, cooling and weighing were repeated until successive weights did not vary by more than 1 mg. The blank was determined using the same procedure without sample. The fat content of feed and resultant powder was calculated by the following formula.

$$\text{Fat (\%)} = \frac{(W_2 - W_1) - (W_4 - W_3)}{W_0} \times 100$$

Where,

W₀= Weight in g of sample

W₁= Weight in g of empty flask for sample

W_2 = Weight in g of empty flask + extracted matter

W_3 = Weight in g of empty flask for blank

W_4 = Weight in g of empty flask + extracted matter for blank

3.6.1.3.2 Crude Fat (AOAC, 2005) for flour, biscuit & muffins

Fat in the product was determined by the ether extract method using the SOCS PLUS instrument. 2-gram moisture-free sample was quantitatively transferred to an extraction thimble. The beakers were washed and dried in a hot air oven at 105°C. The beakers were then cooled in the desiccator for about 5 minutes. The weight of the empty beakers was taken (W). petroleum ether (B.P.40-60°C) was poured inside each beaker and then these beakers were attached to the SOCS PLUS assembly. Extraction was done at 160°C for 90 minutes. The extracted fat was collected in previously weighed extraction beakers and took out the beakers from SOCS assembly. The beakers were dried in oven at 105 °C for 30 minutes and then they were cooled in a desiccator and weighed.

$$\text{Fat, \% by weight} = \frac{(W_1 - W)}{W_2} \times 100$$

Where,

W =weight of extraction beaker

W_1 = weight of extraction beaker +extracted fat after drying

W_2 = weight of the sample

3.6.1.4 Protein

Digestion: In a clean and dry Kjeldahl flask (500 mL), 5-10 boiling aids, nitrogen-free 2.7 g potassium sulphate, 0.3 g copper sulfate (nitrogen-free) and 1 ± 0.1 g sample (s). Any copper sulfate solution, K_2SO_4 or sample residues remaining on the neck of the flask was washed down by the addition of 25 mL of concentrated H_2SO_4 (Strength: 95-98% m/m; nitrogen-free; density approximately 1.84 g/mL). This mixture was then gently mixed followed by digestion at 420°C temperature till a clear solution was obtained.

Distillation: After complete digestion, the digested sample was cooled to room temperature followed by making up the volume to 100 mL using a volumetric flask. The whole content was thoroughly mixed to ensure the complete dissolving of any crystals and cooled to room temperature. The 10 mL of this solution was transferred to an empty Kjeldahl tube.

Immediately after this, the Kjeldahl flask was connected to the distillation apparatus. A flask containing boric acid was connected to the condenser outlet. Boric acid solution (40 g

boric acid was dissolved in hot water and made up to 1 L containing 3 mL of methyl red-bromocresol green indicator) and NaOH (40% w/v) solution lines were connected. Blank was prepared by replacing the sample with 5 mL water and 0.85 g of sucrose.

Titration: The distillate was then removed the from distillation assembly and titrated against 0.1N hydrochloric acid. The appearance of slight violet color indicated the end point. The titer volume was noted. Initially one reading was taken for blank.

The protein content was calculated using following equation and conversion factor:

$$\text{Nitrogen content (\%)} = \frac{1.4007 \times (V_s - V_b) \times \text{Normality of HCl}}{\text{Weight of sample}} \times 100$$

$$\text{Protein content (\%)} = \% \text{ nitrogen content} \times F$$

Where,

V_s = Volume in mL of the standard hydrochloric acid used for the sample

V_b = Volume in mL of the standard hydrochloric acid used for blank

N = Normality of HCl (0.01N)

W = Mass of test portion in g, expressed to nearest 0.1 mg

F = Conversion factor for nitrogen to protein e.g., 6.38 (for milk products) & 5.40 for flour

3.6.1.5 Ash

The ash content was determined as per (AOAC, 2005). Approximately 3 g of sample was accurately weighed into a clean, dry silica crucible. The material was incinerated on a heater till it become smoke free and ignited in a muffle furnace at 550 ± 10 °C until light grey ash resulted, then cooled in a desiccators and weight soon after reaching room temperature. The samples were analysed in triplicate. The ash content was calculated using the following equation:

$$\text{Ash content (\%)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where,

W_1 = Weight (g) of empty crucible

W_2 = Weight (g) of empty crucible + sample

W_3 = Weight (g) of incinerated sample

3.6.1.6 Acid Insoluble Ash

About 3 g of the biscuit powder was weighed accurately in the dish and incinerated in the muffle furnace at 550 ± 10 °C until light grey ash is obtained. The dish was removed from the furnace and allowed to cool at room temperature. 25 ml of the hydrochloric acid (5 M) was added to the dish, covered with a watch glass and heated in the water bath for 10 minutes. The contents were mixed with the tip of a glass rod and filtered through Whatman filter paper No. 42 or its equivalent. The filter paper was washed with water until the washings were free from acid and tested with a blue litmus paper. The washed filter paper was returned to the dish for incineration in the muffle furnace as above. The dish was cooled in a desiccator and weighed. Again, the dish was ignited for half an hour in the furnace, cooled and weighed. This operation was repeated until the dish had a constant weight, the difference between successive weighing being less than 1 mg. 25 ml of the hydrochloric acid was filtered through a blank filter paper, washed and weighed it as in the case of acid insoluble ash. Its weight was subtracted from the weight of insoluble ash of the sample.

$$\text{Acid insoluble ash (\% by weight)} = \frac{100 (W_1 - W)}{W_2}$$

Where,

W = weight in g of empty dish in which the sample is taken for ashing

W_1 = weight in g of the dish containing acid insoluble ash

W_2 = weight in g of the sample

3.6.1.7 Crude fiber

Crude fiber determination was done as per the method described in AOAC (1995). Two-gram fat-free sample (W) was taken and transferred in a spoutless beaker containing 200 ml of 1.25 % H_2SO_4 and boiled for 30 minutes. After 30 minutes, the flask was removed and the solution was filtered through Buchner funnel (Whatman NO.54) and the residue was washed with hot distilled water. The residue was then boiled in 1.25 % NaOH solution for exactly 30 minutes. After 30 minutes of boiling, the contents were filtered through Whatman NO.54 and washed with hot distilled water using Buchner funnel under gentle suction. The residue was collected in a crucible dried in oven at $105^\circ C$ for 3-4 hours or till constant weight. It was cooled in desiccator and weighed (W_1) and this was incinerated in a muffle furnace at $550 \pm 10^\circ C$ for 3-4 hours. Allow to cool to a room temperature and final crucible weight was noted down (W_2). The crude fibre was calculated using the following formula.

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

Where,

W- Weight of sample

W₁- Weight of crucible + fiber residue

W₂ – Weight of crucible with ash

3.6.1.8 Lactose

Lactose content of ghee-residues was determined by difference i.e., by subtracting protein, fat and ash from their respective TS contents using following formula:

$$\text{Lactose (\%)} = \text{TS (\%)} - \text{Fat (\%)} - \text{Protein (\%)} - \text{Ash (\%)}$$

3.6.1.9 Bulk density

Loose bulk density (LBD) and packed (tapped) bulk density (PBD) of ghee residue were estimated as per the procedure described by Sjollema (1963). A 100 mL graduated cylinder of tare weight was taken. A funnel was placed over a cylinder opening and ghee residue allowed to flow freely through the funnel up to the 100 mL mark. The weight of the cylinder together with the powder was recorded. The net weight of 100 mL powder was worked out and the results were expressed as loose bulk density (g/mL). Further, the powder was tapped 100 times tap density tester and the resultant volume was recorded. The volume was read in mL and the packed bulk density was expressed as g/ mL.

3.6.1.10 Flowability

The angle of repose (as a static measure of flow ability) of ghee residue was determined by the method described by Sjollema (1963). A plastic funnel with a narrow stem, cut at right angles, was mounted exactly 2 cm above a piece of butter paper, positioned on a horizontal table. The powder samples were allowed to fall through the funnel in a fine stream at controlled speed, so that a conical heap was formed beneath the funnel. When top of the powder heap touched the end of funnel stem, the powder addition was stopped. The base of the powder heap was outlined with a pencil and the powder was removed. The diameter of the portion marked with pencil was recorded at different points to calculate its radius which was further used to calculate its angle of repose as per following formulae.

$$\tan \Theta = \frac{h}{r}$$

$$\Theta = \tan^{-1} \left(\frac{h}{r} \right)$$

Where,

Θ = Angle of repose

h = Height of stem base (Fixed at 2cm)

r = Radius of the powder heap (cm)

The flowability of ghee residue also determined in terms of their Compressibility index (CI) and Hausner ratio (HR). The CI and HR of ghee residue calculated using their LBD and TBD or PBD values as shown below

$$\text{Compressibility index, (CI)} = \frac{TBD-LBD}{LBD} \times 100$$

$$\text{Hausner ratio, (HR)} = \frac{TBD}{LBD}$$

Where,

TBD = Tapped bulk density

LBD = Loose bulk density

3.6.1.11 Acidity of Extracted fat

The procedure for the acidity of extracted fat was outlined by IS SP: 18 (Part V) – 1982. 10 g of biscuit powder was weighed and transferred it to the thimble and fat was extracted in a previously weighed beaker. The traces of the residual solvent were removed by keeping the flask in the hot air oven for about half an hour and weighed. The flask was cooled and about 50 ml of mixed benzene-alcohol-phenolphthalein reagent (To 1 litre of distilled benzene added 1 litre of alcohol or rectified spirit and 0.4 g of phenolphthalein. Mixed the content) was added and the contents were titrated to a distinct pink colour with the potassium hydroxide (0.05 N) solution taken in a 10 ml micro burette. A blank titration was also made of the 50-ml reagent.

$$\text{Acidity of Extracted fat \% oleic acid} = \frac{1.41(V - B)}{W_1 - W} \times 100$$

where,

V = Volume of 0.05 N potassium hydroxide

B = Blank Titre value

W_1 = Weight in g of Soxhlet flask containing fat

W = Weight in g of empty Soxhlet flask

3.6.1.12 Weight loss

The *muffins* were numbered by marking the underside of the mould and were weighed before (W_2) and after baking followed by one hour cooling (W_1). The weight loss upon baking was calculated as follows:

$$\text{Weight loss} = \frac{(W_2 - W_1)}{W_1} \times 100$$

Where,

W_2 = Weight of muffin batter before baking

W_1 = weight of muffin after baking

3.6.1.13 Specific gravity (SG) of the batter

The SG of the raw batter was measured with a small cup of known volume (Baixauli *et al.*, 2008). It was determined gravimetrically by dividing the weight of this known volume of batter by the weight of an equal volume of water. The measurements were made in triplicate.

3.6.1.14 Water activity (a_w)

Water activity (a_w) of dough & products of biscuit and muffins were measured using a water activity meter. The instrument measures water activity of product with accuracy @ 0.003 at 25°C. AquaLab operates on the principle of chilled mirror dew point technique to measure aw of sample. Firstly, the instrument was calibrated by placing the dry charcoal into the sample tray to absorb the internal moisture of the chamber. The sample was quickly transferred into the sample tray to prevent moisture gain. The tray was placed in the instrument and the chamber sealed for measurement. The readings were taken in triplicates.

3.6.1.15 Hunter-Lab color value

A Tristimulus spectrophotometer Hunter Lab model Colour Flex[®] (MiniScan XE plus, Hunter Associates Laboratory Inc. Reston, Virginia, U.S.A.) along with the software (version 4.10) was used to measure the color of dough, batter biscuits & muffins and the results were expressed in terms of the CIE-LAB system. The instrument was standardized to colorimeter colour. Before the test, the instrument was calibrated with standard black and white tiles as specified by the manufacturer (i.e., L^* 50.83, a^* -26.27 and b^* 12.12). The light source was dual beam xenon flash lamp. Measurements were then made on the sample taken in a glass sample cup (10 cm height and 6 cm diameter) supplied with the instrument by filling it to a fixed level (up to 3 cm) for each sample. Data were received through the software in terms of L^* (lightness), ranging from 0 (black) to 100 (white), a^* (redness),

ranging from +60 (red) to -60 (green), and b^* (yellowness), ranging from +60 (yellow) to -60 (blue) values.

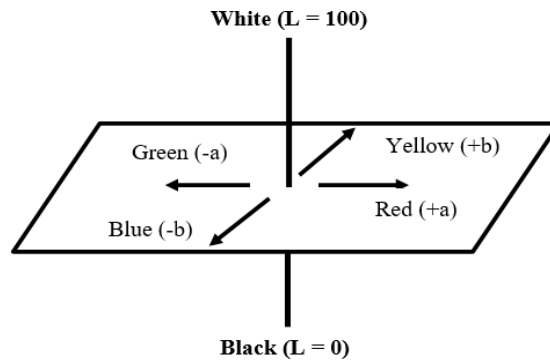


Figure 3.3: Hunter Lab color space

During color measurement care was taken to avoid breaking of the sampling cup. Three random readings of color per sample were recorded and averaged. Further the values of L^* , a^* & b^* were used to calculate the browning index of a biscuit (Isleroglu *et al.*, 2012).

$$\text{Browning index} = \frac{[100 \times (\frac{a+1.79L}{5.645L+a-3.012b} - 0.31)]}{0.17}$$

3.6.1.16 Spread ratio

The biscuits were physically evaluated by measuring their thickness and diameter. Thickness of biscuits was determined by piling six biscuits and then taking their average value. Similarly, diameter was measured of six biscuits and then taking their average value. Spread ratio was calculated by taking ratio of diameter to thickness.

3.6.2 Texture analysis

3.6.2.1 Texture Profile Analysis measurement of biscuits dough

The TA-HD plus texture analyser was switched on and the computer was linked to it. The Texture exponent 32 program was opened. In the Texture exponent 32 program TA settings were selected and the library option was selected in the TA settings. In the library option TPA was selected under special tests

The project settings were entered as follows:

- Pre-test speed – 2.0 mm/sec
- Test speed – 2.0 mm/sec
- Post-test speed - 5.0 mm/sec
- Target mode – distance

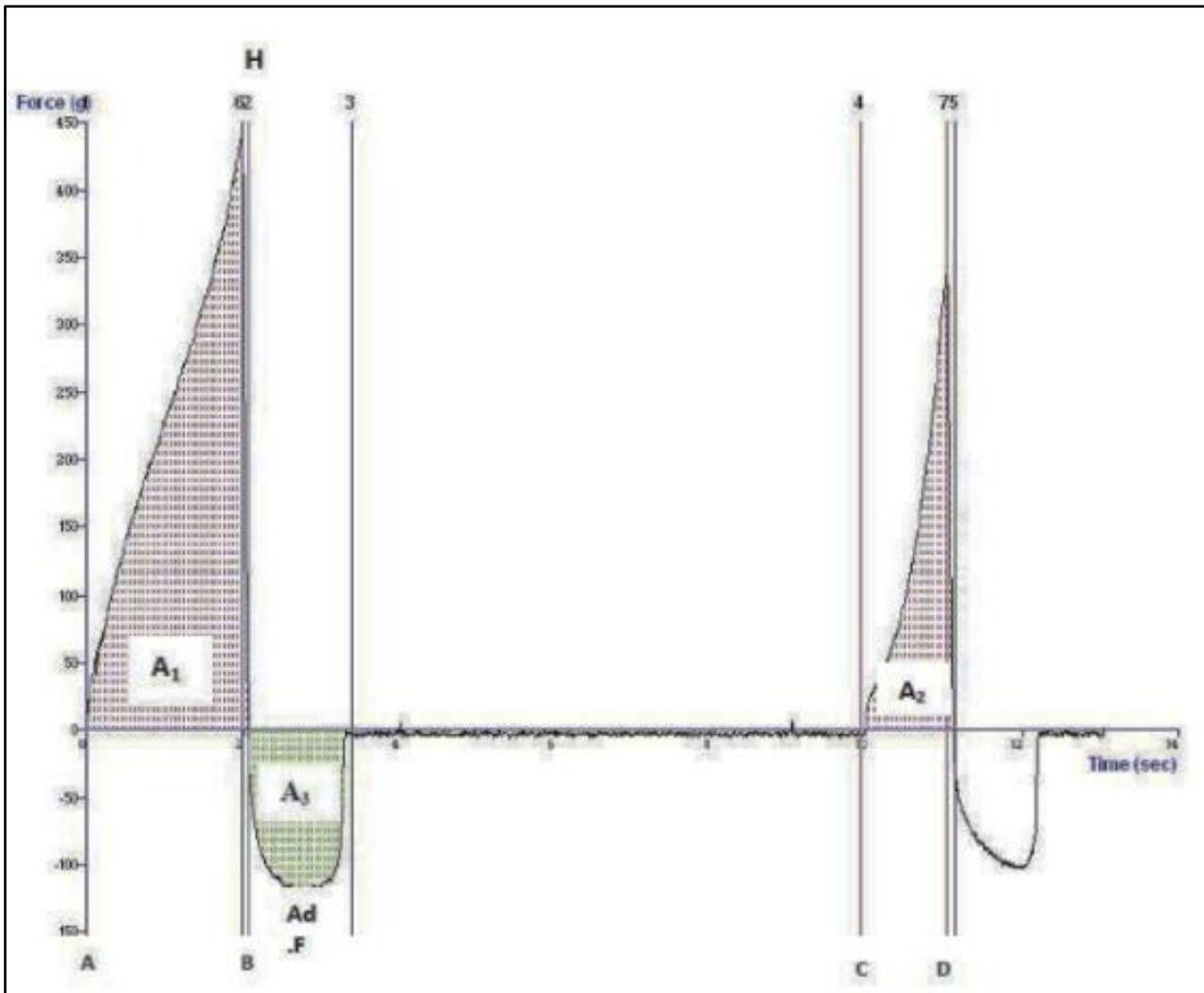
- Distance – 37.50 mm
- Time – 5.0 sec
- Trigger type - Auto (force)
- Trigger force – 0.5g
- Break mode - Off
- Tare mode – Auto
- Advanced options - On
- Control oven - Disabled
- Frame deflection mode - Off (XT2 compatibility)

Accessory: P/75 plunger probe

The probe was calibrated to a distance of 50mm, above the top of the container or the sample surface. The sample of dough tempered to about 25 °C was cut into pieces of 25 mm x 25 mm x 25 mm size. The sample was positioned centrally over the platform and the computer was allowed to execute the program by activating ‘run a test’ option, then the sample was compressed by the plunger twice in a gap of 5 sec to yield a force – time curve. The height of the force peak on the first compression cycle (first bite) is the value of hardness (F). The ratio of the positive force under the second and first compressions (A2/A1) is cohesiveness while the ratio of the time difference between D-E to the time difference between A-B is springiness as shown in the curve. The negative peak gives us the adhesive force and the area of the negative peak gives us the adhesive force. Resilience is the ratio of the area between B-C and A-B. Gumminess and Chewiness are derived parameters calculated as follows:

Gumminess: Hardness x Cohesiveness

Chewiness: Gumminess x Springiness= Hardness x Cohesiveness x Springiness



H = Hardness = Maximum value on first peak (Newton); Cohesiveness = Area of second peak / Area of first peak = A_2/A_1 ; Adhesiveness = A_3 = Area of negative peak (N.s); Adhesive force = Maximum value on first negative peak (Newton); Springiness = Time difference between C & D / Time difference between A & B;

Figure 3.4: Typical Texture Profile Analysis Curve

3.6.2.2 Texture profile analysis of muffins

Texture profile of muffins was analysed by TA-HD plus texture analyser (Stable Micro System). Estimations were done in triplications. All the textural analysis for muffins was done on Heavy Duty Platform (HDP/90) using stainless steel probe (75 dia. Compression platen -p/75). Analysis was carried out by two compression cycles using the probe. The muffins were cut horizontally at the height of the mould, the upper half was discarded and the 2.5 cm-high lower halves were removed from the mould. A double compression test was performed to a height of 1.25 cm (50%compression) with a 75 mm diameter 5 s waiting time

between the two cycles. The parameters obtained from the curves were hardness, springiness, cohesiveness, gumminess, chewiness. The texture analyser was set to

- Mode- Measure force in compression
- Option - Return to start
- Pre-test speed- 2.0 mm /sec
- Test speed- 2.0 mm/sec
- Post-test speed- 2.0mm/sec
- Trigger type - Auto
- Data acquisition rate - 200.00 PPS
- Force - 0.98 N
- Time interval - 5 Sec
- Load cell - 25 kg
- Compression- 50% strain
- Break mode - Off
- Tare mode – Auto
- Advanced options - On
- Control oven - Disabled
- Frame deflection mode - Off (XT2 compatibility)

The textural parameters were worked out from the force-time curve thus obtained for each sample with force experienced by the probe on Y-axis and time on X-axis. Peak force at the end of first compression cycle indicated hardness/firmness of the sample while other parameter was calculated and given by the software of the equipment.

3.6.2.3 Cutting strength

The sample biscuits were evaluated for hardness using Texture analyser TA-HD plus (Stable Microsystems, USA) fitted with a 50 kg load cell. The individual samples of biscuits were placed on the platform and the blade was attached to the cross head of the instrument. The test conditions maintained were as follows

- Pre-test speed- 2mm/s
- Test speed- 3mm/s
- Post-test speed- 10mm/s and
- Distance- 10mm

The absolute peak force of the resulting curve was considered as cutting strength of the biscuit.

3.6.3 Dynamic rheological Properties

3.6.3.1 Rheological properties of the dough

Dough rheological characterization was performed at 25 ± 0.1 °C with a rheometer (model; MCR52; make: Anton Paar, Germany) equipped with a 50-mm diameter plate–plate sensor geometry with a 1-mm gap between the plates was employed. The dough samples were placed on the lower plate and the upper plate was dropped until the distance between plates reached to 1 mm. During loading, the developed residual stress was removed by providing 5 min resting time to sample. The extra dough was trimmed and the exposed edge of the dough sample was covered with a thin layer of liquid paraffin to avoid moisture loss during experiments. To measure dynamic viscoelastic properties, initially, the linear viscoelastic range of the samples was obtained with a strain sweep (0.01-100%) at a constant frequency of 1 Hz. Thereafter, dynamical oscillatory frequency sweep test at 25°C using the parallel plate geometry (PP50) in controlled-strain mode at a frequency range from 0.1 to 100 Hz was conducted at constant strain obtained from amplitude sweep. The storage modulus (G') and loss modulus (G'') as a function of frequency were continuously accessed throughout the test.

Creep test on dough was conducted by using the same rheometer. In creep test (Abebe *et al.*, 2015), the dough sample was placed at 25°C and fixed shear stress of 50 Pa was applied for 60 s in the linear viscoelastic range. Thereafter, during the recovery phase, the shear stress was removed promptly and the sample was rested for 180 s to retrieve the elasticity (instantaneous and retarded) of the deformation. The Rheoplus/32 software of the Rheometer calculated the creep and creep recovery parameters. The maximum compliance ($J_{C_{max}}$) value reached in the creep phase for 60 s, which corresponds to the maximum deformation and the maximum compliance value at the end of the recovery phase ($J_{r_{max}}$), related to partial reformation after stress removal.

$$\text{Recovery (\%)} = \frac{J_{r_{max}}}{J_{C_{max}}}$$

All the rheological experiments were performed in triplicates.

3.6.3.2 Rheological properties of the batter

During the rheological determinations, special attention was paid to maintaining samples with the same thermo-mechanical history before testing. Accordingly, the batters

were all kept at $25 \pm 1.0^{\circ}\text{C}$ for 1 h after batter preparation before the rheological test. During loading, the developed residual stress was removed by providing 5 min resting time to sample. The extra batter was trimmed and the exposed edge of the batter sample was covered with a thin layer of liquid paraffin to avoid moisture loss during experiments. To measure dynamic viscoelastic properties, initially, the linear viscoelastic range of the samples was obtained with a strain sweep (0.01-100%) at a constant frequency of 1 Hz.

3.6.3.2.1 Flow properties

The flow properties of the muffin batters were studied using a rheometer (model; MCR52; make: Anton Paar, Germany) equipped with a 50-mm diameter plate–plate sensor geometry with a 1-mm gap between the plates was employed, which was considered large enough with regard to the starch granule size (maximum size around 35 μm). A continuous ramp was applied and apparent viscosity was measured as a function of shear rate over the $1.0\text{--}1000\text{ s}^{-1}$ range, taking 100 points linearly in time at 25°C . Three replicates were conducted for control and treatment batters.

3.6.3.2.2 Viscoelastic properties

Linear viscoelastic properties were studied with a rheometer (Anton par) equipped with a PP50. stress sweep tests (1 Hz at 25 and at 85°C) were made to determine the linear viscoelastic region of all samples. Temperature dependence was studied by applying a temperature sweep at 1 Hz from 25 to 85°C at a heating rate of $2^{\circ}\text{C}/\text{min}$. During the temperature sweep the applied stress was always self-adjusted in order to keep measurements throughout the range of temperatures studied within the linear viscoelasticity regime, i.e., taking into account the critical strain for the onset of non-linear response. Three replicates of each oscillatory dynamic test were conducted for each kind of batter.

To simulate the effect of heating in the batter structure, temperature sweeps were performed from 25 C to 95°C at a heating rate of $2.0^{\circ}\text{C}/\text{min}$ and a strain amplitude of 0.05 %. The strain applied was selected to guarantee the existence of a linear viscoelastic response. Liquid paraffin was applied to the exposed surfaces of all the samples. Three replicates from different batches of each formula were measured. Evolution of viscosity with temperature was studied applying a shear rate of 1 s^{-1} from 25 to 90°C at a heating time of $2^{\circ}\text{C}/\text{minute}$.

3.6.4 Sensory evaluation

The samples for evaluation were coded appropriately before serving the samples to the judges for sensory evaluation.

3.6.4.1 Biscuit

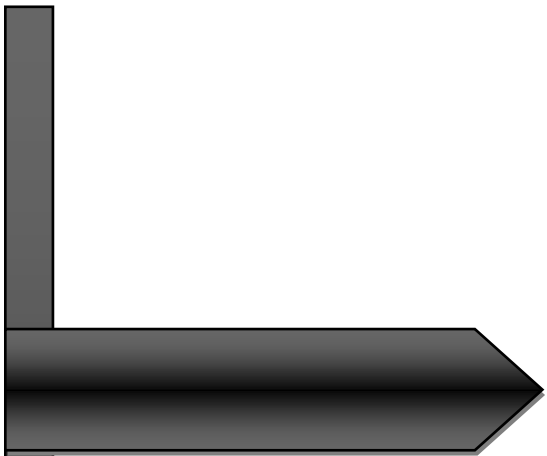
The organoleptic quality of bajra bases biscuits were evaluated by an expert panel of judges using a linear intensity score card. Biscuits were analysed for appearance, flavour, texture and overall acceptability. The intensity of the perceived attributes and overall acceptability was marked in 1 – 10 intensity scale (Nandagopal, 2017). A sensory score card has been given in the Appendix A.

3.6.4.2 Muffin

The Ghee residue powder added muffin samples were analysed for sensory parameters evaluated by an expert panel of judges on 9-point hedonic scale wherein a score of 1 represented 'dislike extremely' and score 9 represented 'like extremely'. muffin were flavour, sweetness, body and texture, colour and appearance and overall acceptability. The panellists were asked to score for the following parameters: a) Colour and appearance b) Body and texture c) Flavour d) Sweetness e) Overall acceptability

3.7 Statistical analysis

The data obtained from various experiments was recorded as mean \pm Standard deviation and subjected to statistical analysis to arrive at valid and meaningful influences. Data was analysed using one way-ANOVA and two -way ANOVA. Least significant differences were calculated by the Tukey test and the significance at $p < 0.05$ was determined. These analyses were performed using SPSS for Windows Version 26.0



CHAPTER 4



RESULTS AND DISCUSSION

Results and Discussion

The purpose of the current study was to develop pearl millet-wheat flour-based biscuits and muffins by replacing bakery fat with ghee-residue &/or GRP (in biscuits) and whey protein concentrate (WPC) with ghee-residue powder (in muffins). The effect of replacement of bakery fat and WPC with ghee-residue (GR) and/or ghee-residue powder (GRP) on the rheological characteristics of biscuit dough and muffin batter was investigated. Further, the effect of baking powder composition, its level and dough resting period were also monitored for the standardization purpose. These muffins and biscuits were assessed for their physical, textural, sensory and physicochemical properties. The findings of the investigations are presented here under: -

4.1 Preliminary analysis

4.1.1 Proximate analysis of flours

Proximate analysis of refined wheat flour, pearl millet flour, GR and GRP were performed to investigate the effect of flour, GR, and GRP on the quality attributes of biscuits and muffins.

The data for various compositional parameters of pearl millet flour (PMF) and refined wheat flour (RWF) has been given in Table 4.1. The proximate composition of pearl millet flour and refined wheat flour differed significantly ($p < 0.05$). The moisture content of pearl millet flour ($p < 0.05$) was lower due to the lesser moisture in the grain as the grains were dried. Further abrasion forces during the milling generate heat which also results in decreased moisture content. Sharoba (2021) reported similar proximate composition of pearl millet flour as obtained during the present investigation. The moisture content of the flour varied between 6.53 % to 9.33 %; whereas the fat, protein and ash content of pearl millet flour and refined wheat flour were 5.62, 9.14 %, 1.66 %, and 1.67 %, 9.84%, 0.65 %, respectively. The higher ash content in PMF might be due to their richness in mineral content and the lower ash content in refined wheat flour could be due to flour being mostly derived from the endosperm. Moreover, RWF was procured from the market and subjected to air-classification which might have resulted in the removal of mineral rich bran fraction. The crude fibre content of pearl millet was 1.96 %, which was higher ($p < 0.05$) as compared to other cereal flours. Flour contains starch, protein, fat and water which play a vital role in biscuit and muffin making. From the perspective of the baker, moisture content should be as low as feasible; as the flour can be kept for a longer duration and it is economical as well. Furthermore, higher moisture

levels in the flour may lead to higher chances of insect and mould infestation during storage. Higher moisture levels in flour, especially in millet flour, favour enzymatic activities. Protein and starch have a major impact on the water absorption capacity of flour while, protein has a direct impact on the biscuit's hardness. The chemical constituents of flour absorb moisture to a variable level and even native and damaged starches too differ in water absorption capacity. Gaines (1988) claimed that as flour moisture content was enhanced, the dough flow, adhesion and diameter of biscuit increased. The protein levels of flour for biscuit making should be around 8-11 % because gluten must form a minimal network for optimum sheeting and dough forming. Gluten in refined wheat flour, on the other hand, may pose allergenicity and manifest in the form of celiac disease, an immune-mediated condition. Hence, to overcome the problem of wheat protein allergy and to develop nutrient-rich biscuits, replacing refined wheat flour with other cereal or millet flours may be an ideal option. In addition, the amount of ash in flour indicates the degree of purity of flour in terms of bran fragments. The higher the ash, the more the bran content and the bran content further affects the colour of the product. Also, the higher fibre content in dough or batter retains a lesser amount of gas during the different stages of processing thereby, resulting in lower volume.

Table 4.1: Proximate analysis of pearl millet and refined wheat flour

| Parameter | Pearl millet flour | Refined wheat flour | C.D 0.05 |
|-------------|--------------------------|--------------------------|----------|
| Moisture | 6.53 ^a ± 0.63 | 9.33 ^b ± 0.20 | 0.81 |
| Fat | 5.62 ^b ± 0.07 | 1.67 ^a ± 0.02 | 0.18 |
| Protein | 9.14 ^b ± 0.05 | 9.88 ^a ± 0.02 | 0.07 |
| Ash | 1.66 ^b ± 0.02 | 0.65 ^a ± 0.03 | 0.05 |
| Crude Fiber | 1.96 ± 0.08 | ND | ND |

C.D (critical difference) at 5% levels of significant; ND- Not detected

^{ab} Mean with a different superscript in different columns of a row differ significantly (p<0.05)

4.1.2 Proximate analysis of GR and GRP

Fresh ghee-residue sample procured from Model Dairy Plant, NDRI, Karnal was utilized as such and also converted into ghee-residue powder (GRP) as per the method suggested by Thalor (2021). The GR and GRP were analysed for their chemical composition and the results have been presented in Table 4.2. Thalor (2021) reported similar results in terms of chemical composition for GR and GRP. As evident from the results, GRP had higher values (p<0.05) for total solids, fat, protein ash and lactose than GR (on a fresh weight basis).

Table 4.2: Proximate and physico-chemical analysis of GR and GRP

| Parameter | | GR | Ghee residue powder | C.D _{0.05} |
|---------------------------|------------------------------|---------------------------|---------------------------|---------------------|
| Total solid (%) | | 28.19 ^a ± 0.37 | 99.08 ^b ± 0.01 | 0.46 |
| Fat (%) | | 8.18 ^a ± 0.42 | 46.09 ^b ± 0.44 | 0.69 |
| Protein (%) | | 12.18 ^a ± 0.38 | 31.18 ^b ± 0.13 | 0.49 |
| Ash (%) | | 1.49 ^a ± 0.01 | 4.91 ^b ± 0.03 | 0.17 |
| Lactose (%) | | 6.32 ^a ± 0.21 | 16.55 ^b ± 0.45 | 0.64 |
| Bulk Density (g/mL) | | NA | 0.43 ± 0.00 | - |
| Tapped density (g/mL) | | NA | 0.53 ± 0.01 | - |
| Flow Properties | Angle of Repose (θ) | NA | 36.38 ± 0.94 | - |
| | Compressibility index (%) | NA | 18.44 ± 0.54 | - |
| | Hausner ratio | NA | 1.22 ± 0.00 | - |
| Reconstitution properties | Wettability (s) | NA | 34.4 ± 1.96 | - |
| | Dispersibility (%) | NA | 54.77 ± 0.90 | - |
| Water activity | | NA | 0.293 ± 0.00 | - |
| Colour values | L* | 38.26 ^a ± 0.13 | 49.37 ^b ± 0.08 | 0.16 |
| | a* | 8.11 ^a ± 0.02 | 10.94 ^b ± 0.01 | 0 |
| | b* | 29.56 ^a ± 0.06 | 34.44 ^b ± 0.25 | 0.18 |

C.D (critical difference) at 5% level of significance; NA-Not Applicable

^{ab} Means with a different superscript in different columns of a row differ significantly ($p < 0.05$)

Bulk density indicates the flow properties of the powders and it is also required for designing of package. Tapped density is often measured because of better reproducibility and it is required for the determination of flowability. The ratio of tapped density W/V_{50} to fluffy density (W/V_0 g/ml) is known as the Hausner ratio. Hausner ratio greater than 1.25 reflects the better flow properties of the powder and a poor flow possesses values less than 1.5. In the present study, GRP was found to contain slightly better flow properties ($p > 0.05$). The compressibility index is also an indicator of flow characteristics and is expressed as the ‘Carr index’. The compressibility index is an indirect measure of bulk density, size and shape, surface area, moisture content and cohesiveness of any powdered material. A value of more than 18 indicates poor flow properties. Significantly higher fat content ($p < 0.05$) in GRP might be the prime reason for poor flow characteristics. Likewise, the re-constitutional properties of

the GRP were also inferior in comparison to other dairy powders. Further, wettability is the characteristic of the material to maintain contact with liquid and depends on adhesiveness and cohesiveness. The wettability of GRP powder is appreciable however; the dispersibility value is lower ($p < 0.05$) as compared to other dairy powders. Among the colour parameters, lightness, redness and yellowness values of GRP were significantly higher ($p < 0.05$) than the GR (Table 4.2).

4.2 Physical, Textural and Rheological parameters of GR substituted biscuit dough

4.2.1 Colour and water activity of dough

The colour and water activity of various doughs are depicted in Table 4.3. The lightness and yellowness of the dough were reduced with the substitution of GR due to the presence of Maillard reaction products in GR. The lightness and yellowness values of the dough were maximum for WBD50 and the lowest for WBDGR25 ($p < 0.05$), whilst the redness of the dough increased from -1.09 (WBD50) to 5.22 (WBDGR25) with increasing the levels of GR. Kumar *et al.* (2015) reported a similar trend of L^* , a^* & b^* values with the addition of a multigrain mix. GR substitution slightly enhanced the water activity and was significantly higher ($p < 0.05$) only at 10% GR addition.

Table 4.3: Effect on colour and water activity of biscuit dough upon GR substitution

| Dough sample | L^* | a^* | b^* | Water activity |
|--------------|-------------------------|-------------------------|-------------------------|-------------------------|
| WBD50 | 45.44±0.34 ^e | -1.09±0.23 ^a | 19.22±0.29 ^d | 0.847±0.00 ^a |
| WBDGR10 | 37.31±0.21 ^d | 1.76±0.62 ^b | 18.44±0.45 ^c | 0.872±0.00 ^b |
| WBDGR15 | 35.73±0.45 ^c | 3.14±0.41 ^c | 16.01±0.61 ^b | 0.839±0.01 ^a |
| WBDGR20 | 32.80±0.21 ^b | 4.56±0.47 ^d | 15.80±0.15 ^b | 0.851±0.01 ^a |
| WBDGR25 | 29.67±0.56 ^a | 5.22±0.05 ^e | 13.23±0.30 ^a | 0.849±0.01 ^a |

*Data are presented as Means ± S.D (n=3)

^{abcde} Means with a different superscript in different rows of column differ significantly ($p < 0.05$)

Note: WBD50- Dough from RWF: PMF (50:50) with 100% shortening, WBDGR10- 10 % fat replaced with ghee residue, WBDGR15- 15 % fat replaced with GR, WBDGR20- 20 % fat replaced with GR & WBDGR25- 25 % fat replaced with GR

There was no consistent trend for water activity of dough with added GR. Variation in water activity might be due to the fact that fat was replaced with GR; if there was enough fat available in the dough, it would coat flour particles and water would be available in free form (Davidson, 2016).

4.2.2 Textural properties of dough

Fat content is mainly responsible for the textural properties of biscuits, as it is involved in tenderness and air incorporation (O'Brien *et al.*, 2003). Bakery fat substitution with GR resulted in a decrease in hardness and gumminess linearly ($p < 0.05$) except for resilience, which increased non-significantly ($p > 0.05$). The other textural parameters also decreased but there was no consistent trend for adhesiveness, cohesiveness, springiness and chewiness and the dough made with GR @15 per cent exhibited the lowest values ($p < 0.05$) for the above-mentioned parameters. Additionally, as fat was reduced, the adhesiveness and resilience increased in the dough. Further, the hardness of the dough will tend to decrease the spread ratio of the biscuits (Manohar & Rao, 2002). In comparison to other dough samples, the WBD50 dough sample had the maximum hardness ($p < 0.05$). It was also observed from the physical characteristics of these doughs (Table 4.9), that the spread of the biscuits rose as the amount of GR increased. According to Umesha *et al.* (2015), the porosity of the biscuit dough affects the hardness. As a result, substituting fat with GR lowered the hardness. However, Nirmala *et al.* (2011) observed that there was a reduction in the springiness and gumminess value of cookie dough with the inclusion of fenugreek and flax seed powder, which was mainly attributed to gluten dilution.

Table 4.4: Effect of GR substitution on Textural properties of biscuit dough

| Dough sample | Hardness (N) | Adhesiveness (g.s) | Springiness (mm) | Cohesiveness | Gumminess (N) | Chewiness (N.mm) | Resilience |
|--------------|--------------------------|-----------------------------|-------------------------|-------------------------|------------------------|------------------------|------------------------|
| WBD50 | 17.56±2.29 ^d | -1069.14±46.33 ^a | 0.24±0.03 ^c | 0.24±0.03 ^{bc} | 4.18±0.10 ^c | 1.01±0.12 ^b | 0.02±0.00 ^a |
| WBDGR10 | 14.97±0.79 ^{cd} | -870.32±317.64 ^a | 0.26±0.04 ^c | 0.26±0.04 ^c | 3.08±0.40 ^b | 0.81±0.23 ^b | 0.02±0.00 ^a |
| WBDGR15 | 11.85±0.20 ^{bc} | -17.08±0.34 ^b | 0.04±0.01 ^a | 0.13±0.01 ^a | 2.00±0.06 ^a | 0.09±0.01 ^a | 0.03±0.00 ^b |
| WBDGR20 | 10.14±1.06 ^{ab} | -230.49±16.91 ^b | 0.08±0.01 ^{ab} | 0.20±0.02 ^b | 1.99±0.06 ^a | 0.16±0.01 ^a | 0.03±0.00 ^b |
| WBDGR25 | 7.28±0.58 ^a | -116.00±9.16 ^b | 0.11±0.01 ^b | 0.23±0.01 ^{bc} | 1.61±0.08 ^a | 0.14±0.01 ^a | 0.03±0.00 ^c |

*Data are presented as Means ± S.D (n=5)

^{abcd} Means with a different superscript in different rows of a column differ significantly ($p < 0.05$)

4.2.3 Rheological properties of dough

Knowledge of dough rheology is crucial as it affects biscuit quality and process efficiency (Pareyt & Delcour, 2008). The use of mechanical and heat energy alters the rheological properties of dough. Furthermore, these qualities are controlled by numerous aspects such as mixing methods, mixing equipment, mixing time, type of ingredients and quality, dough temperature, and water content (Manohar & Rao, 1999).

4.2.3.1 Creep test of a dough

Maximum strain refers to the dough's extensibility (Wang & Sun, 2002), it was noted that when the GR levels were increased simultaneously the dough's extensibility decreased. The other dough combinations showed more extensible behaviour than the WBD50 dough. The physical property of dough and its elastic nature were inextricably correlated. If the dough was elastic, the spread of a biscuit will also be less; however, in this study, the elasticity of the dough was reduced and the spread of the biscuit increased (depicted in Table 4.9). The elasticity of the dough was often represented in terms of recovery and percentage recovery. Due to shrinkage/elastic recovery of a dough, higher recovery and percentage recovery tend to diminish the diameter of the biscuit. The highest elastic recovery was due to the high protein content in flour; however, when the GR was incorporated into the dough, it resulted in increased moisture content of the dough; rheological analysis revealed that WBD50 dough had the highest recovery and percentage recovery when compared to all other doughs (Table 4.5). However, the highest rate of recovery is undesirable.

Table 4.5: Effect of GR substitution on rheological characteristics of biscuit dough

| Dough sample | Max. Strain | Recovery | Percentage Recovery |
|--------------|-------------------------|------------|----------------------------|
| WBD50 | 0.03±0.01 ^a | 0.020±0.00 | 63.27 ± 23.35 ^b |
| WBDGR10 | 0.09±0.02 ^b | 0.015±0.00 | 18.52±9.79 ^a |
| WBDGR15 | 0.06±0.02 ^{ab} | 0.016±0.00 | 26.78±14.35 ^{ab} |
| WBDGR20 | 0.05±0.02 ^{ab} | 0.021±0.00 | 39.88±12.67 ^{ab} |
| WBDGR25 | 0.05±0.01 ^{ab} | 0.018±0.00 | 30.98±12.78 ^{ab} |

*Data are presented as Means ± S.D (n=3)

^{ab} Mean with different superscripts in different rows of column differ significantly (p<0.05)

It can be observed in Figure 4.1 that during the creeping stage, the strain grew with time in response to the constant force applied, causing the dough to deform quickly. It was seen that after a certain period, the strain approximated a stable state by attaining equilibrium deformation. When the constant force was removed during the recovery stage, the dough strain was partially recovered from equilibrium deformation to a constant value over time. Furthermore, dough creep–recovery curves demonstrated normal viscoelastic behaviour, integrating viscous fluid and elastic components (Steffe, 1992). The results obtained (Figure 4.1) showed that the 10 % GR substituted sample (WBDGR10) had the highest strain with the lowest recovery and per cent recovery. It signified that the WBDGR10 dough was stiffer than the other dough samples in terms of dough strength.

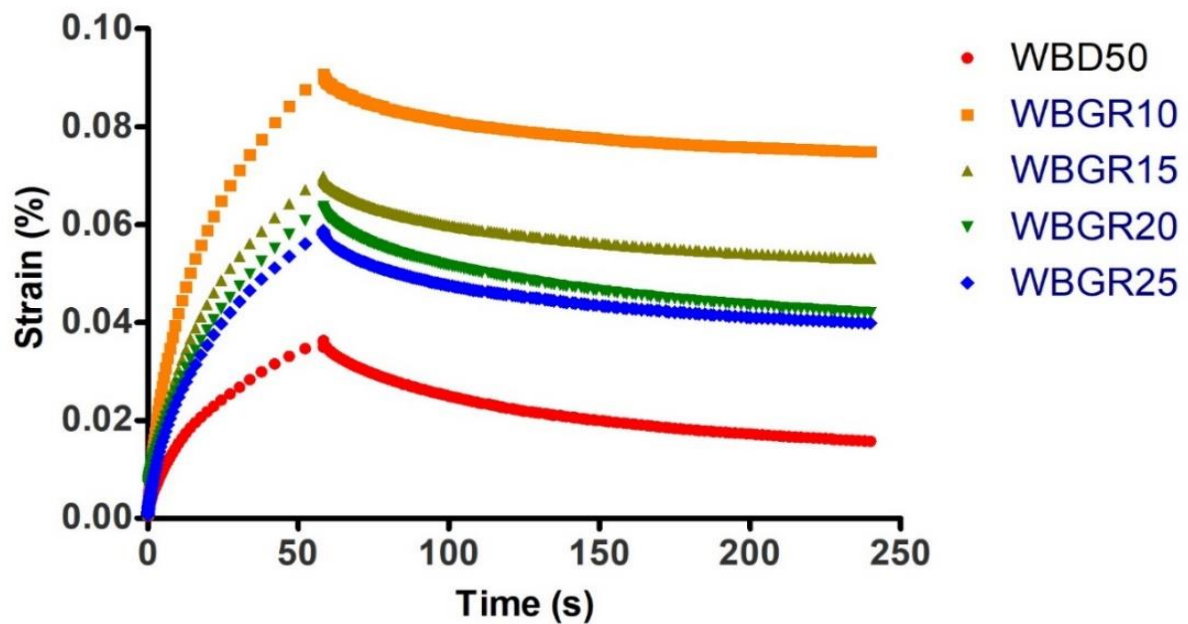


Fig4.1: Creep Recovery of ghee-residue substituted dough samples

4.2.3.2 Frequency sweep of ghee-residue substituted dough samples

Frequency sweep tests were performed in the viscoelastic area acquired from amplitude sweep tests to obtain relevant information from the viscoelastic properties of different dough samples. The elastic or storage modulus (G') expresses the amount of energy stored in the material or recovered during each cycle of deformation. The viscous or loss modulus (G'') estimates the amount of energy lost per cycle of deformation due to viscous dissipation. As shown in Fig. 4.2, the storage modulus G' was larger than the loss modulus G'' for all samples across the entire frequency range, indicating that the examined doughs were more elastic than viscous. Mastromatteo *et al.* (2013) also reported that the durum wheat dough samples were more elastic in nature rather than viscous. The dough sample WBGR15 had the highest G' and G'' values. The WBGR25 dough sample, on the other hand, had the lowest G' and G'' values.

One of the parameters that show the ratio of loss modulus (G'') and elastic modulus (G') is the loss factor ($\tan \delta$) (Jekle *et al.*, 2016). When $\tan \delta$ is less than one, G' is greater than G'' , indicating that elastic properties dominate over viscous properties in the dough (Zhang *et al.*, 2017). $\tan \delta$ was highest for GR substituted to dough sample i.e., WBGR25, indicating that the dough is viscous, and lowest for WBD50, indicating that the dough is elastic, as shown in Fig 4.3. It could be due to an increase in GR levels, and increased water content of the dough samples. Letang *et al.* (1999) observed that increasing the water content softened the dough samples and $\tan \delta$ was usually low in highly structured materials.

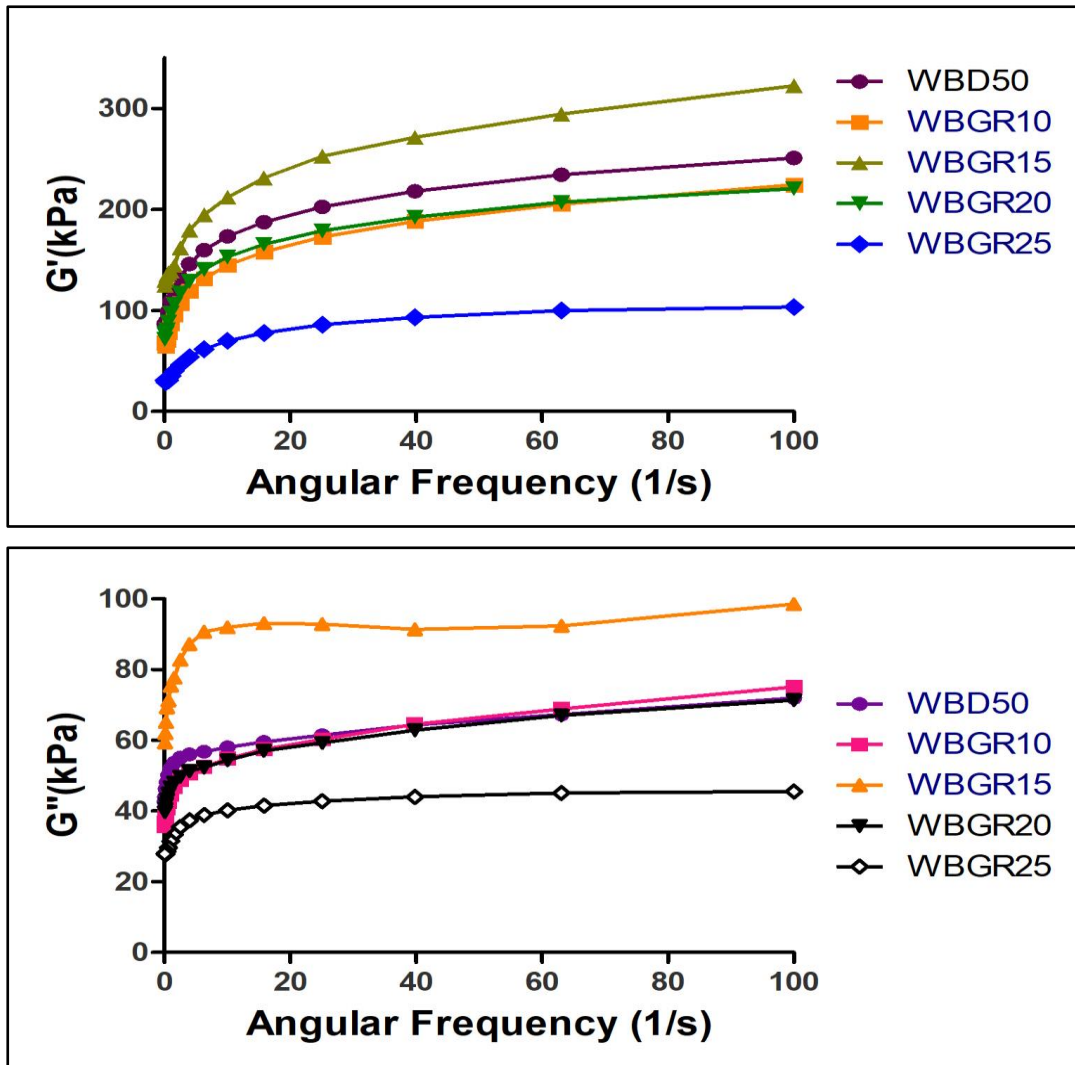


Fig 4.2: Elastic modulus (G') and loss modulus (G'') as functions of frequency for different GR substituted samples; Spectrum measured between 0.1 and 100 Hz

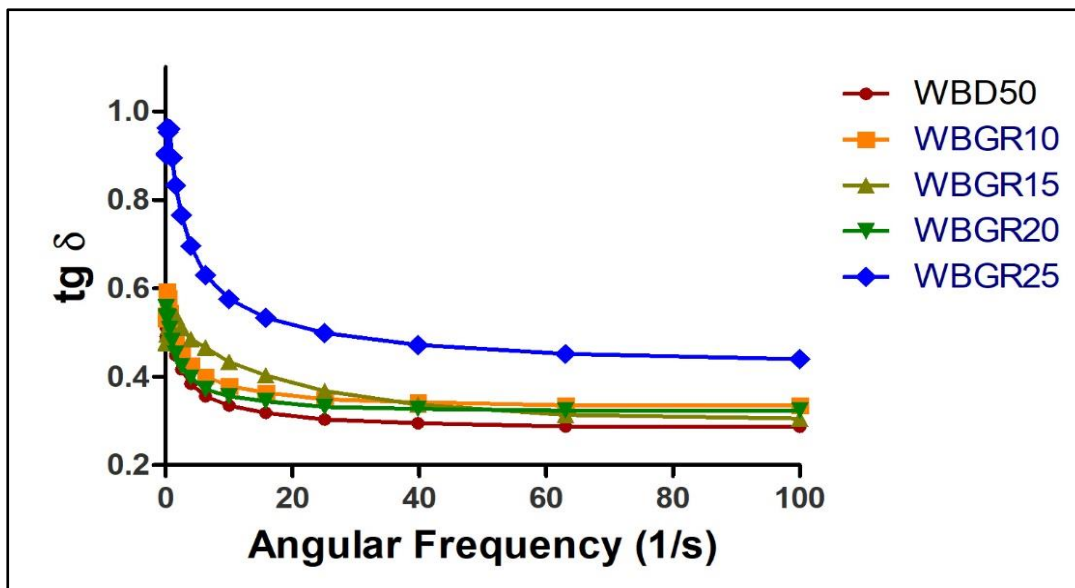


Fig 4.3: Dynamic viscoelastic loss factor with frequency dependence ($\tan \delta$)

4.3 The effect of GR on the quality parameters of biscuits

4.3.1 Effect of different levels of GR on the proximate values of pearl millet-based biscuits

Cookies are low moisture baked foods and higher moisture in cookies may result in a moist and soft texture thereby, lowering the consumer appeal (Chevalier *et al.*, 2000). The moisture percentage of biscuit samples ranged from 0.55% to 1.52%, while the fat content ranged from 18.66 to 22.35%. Fat content reduced ($p < 0.05$) as the GR levels increased in biscuit formulation due to lower fat levels in GR than shortening. Borawake and Bhosale (1996) also reported that GR incorporation led to the decreased fat content in biscuits. However, the acidity of the extracted fat increased slightly, but there was no significant increase in acidity ($p > 0.05$) of extracted fat between the control and biscuit made with 10% GR. GR had little effect on protein content because there was no significant change in protein percentage ($p > 0.05$). Florence *et al.* (2014) reported almost similar protein values for pearl millet cookies. Ash gradually increased with increasing GR levels but there was not much significant difference in the ash content ($p > 0.05$). The detailed values of various dough samples are shown in Table 4.6.

Table 4.6: Effect of different levels of GR on the proximate values of PM-based biscuit

| Treatments | Moisture (%) | Fat (%) | Acidity of extracted fat (% oleic acid) | Protein (%) | Ash (%) | Acid insoluble ash (%) |
|------------|-------------------------|--------------------------|---|-------------|-----------|--------------------------|
| Control | 0.95±0.11 ^b | 22.35±0.83 ^b | 0.30±0.01 ^a | 6.14±0.27 | 1.21±0.04 | 0.062±0.03 ^b |
| 10 % GR | 0.55±0.15 ^a | 21.62±0.81 ^b | 0.31±0.01 ^a | 6.79±0.05 | 1.15±0.04 | 0.016±0.00 ^a |
| 15% GR | 0.66±0.04 ^{ab} | 20.96±1.15 ^{ab} | 0.33±0.02 ^b | 6.86±0.89 | 1.17±0.05 | 0.026±0.00 ^{ab} |
| 20% GR | 1.01±0.10 ^b | 19.21±0.79 ^a | 0.34±0.00 ^b | 6.44±0.80 | 1.19±0.04 | 0.033±0.00 ^a |
| 25% GR | 1.52±0.22 ^c | 18.66±1.90 ^a | 0.35±0.00 ^b | 6.64±0.25 | 1.21±0.09 | 0.042±0.01 ^c |

*Data are presented as Means ± S.D (n=3)

^{abc}Mean with different superscripts in different rows of a columns differ significantly ($p < 0.05$)

Note: control- Biscuit from Wheat: pearl-millet flour (50:50) with 100% shortening, 10% GR- 10 % fat replaced with ghee residue, 15% GR- 15 % fat replaced with GR, 20% GR- 20 % fat replaced with GR & 25% GR- 25 % fat replaced with GR

4.3.2 Effect of different levels of GR on the colour and water activity of pearl millet-based biscuit

The biscuit's colour and water activity are key parameters as colour influences consumer acceptability of a product. Similarly, water activity is critical as it determines the product's shelf life and texture. Table 4.7 shows the colour of the biscuit samples expressed in terms of tri-stimulus characteristics, L^* , a^* , and b^* values. According to the results obtained, colour values for all biscuit samples were significantly different ($p < 0.05$). In Table 4.7, as GR levels increased, the lightness levels increased significantly ($p < 0.05$), while the redness (a^* values) and yellowness (positive b^* values) values were gradually decreasing. Nandagopal (2017) found that increasing the whey levels improved the colour of the multigrain biscuit.

Table 4.7: Effect on colour and water activity of biscuits upon GR substitution

| Treatments | L^* | a^* | b^* | a_w |
|------------|--------------------------|-------------------------|-------------------------|--------------------------|
| Control | 49.51±0.42 ^{ab} | 11.36±0.04 ^b | 27.71±0.16 ^c | 0.183±0.01 ^b |
| 10 % GR | 48.93±0.23 ^a | 11.43±0.13 ^b | 28.33±0.23 ^d | 0.168±0.02 ^a |
| 15% GR | 49.82±0.24 ^b | 11.11±0.05 ^b | 27.49±0.27 ^c | 0.164±0.01 ^{ab} |
| 20% GR | 50.81±0.14 ^c | 09.90±0.06 ^a | 26.40±0.13 ^b | 0.171±0.00 ^a |
| 25% GR | 52.82±0.05 ^d | 09.75±0.25 ^a | 25.42±0.16 ^a | 0.195±0.01 ^c |

*Data are presented as Means ± S.D (n=3)

^{abc} Mean with different superscripts in different rows of columns differ significantly ($p < 0.05$)

Biscuits incorporated with 25% GR had the highest lightness value ($p < 0.05$), whereas the control biscuit had the lowest ($p < 0.05$). The redness and yellowness values of 10% GR biscuits were higher ($p < 0.05$) than those of 25% GR biscuits while water activity was highest ($p < 0.05$) for 25% GR biscuits and lowest ($p < 0.05$) for 15% GR biscuits.

4.3.3 Sensory analysis of GR substituted pearl millet biscuit

Descriptive sensory analysis is a standard tool that provides detailed information about the nature and intensity of sensory attributes as perceived by humans while adjudging food (Omoba *et al.*, 2015). The sensory characteristics of biscuits treated at various levels of GR are shown in Table 4.8. No variations in the overall acceptability of biscuits were observed ($p > 0.05$). However, the control had higher acceptability than the ghee-residue substituted biscuits ($p < 0.05$). Among the GR substituted biscuits, 20% GR biscuits were more acceptable. It was also observed that with the substitution of GR for a pearl millet biscuit, the powdery surface was decreased ($p < 0.05$). Further, biscuits showed no signs of development of rancid and foreign flavour with the substitution of GR. Moreover, the sweetness of a product decreased but there was no significant difference ($p < 0.05$). Crispness, coarseness,

adhesiveness and chewiness were found to be higher with no significant difference ($p>0.05$). The hardness of a product was increased due to fat replacement with GR which could be significantly correlated with the reduction in the tenderness of a product leading to an increase in the hardness. The product images were shown in the Figure 4.4.

Table 4.8: Effect of substitution of GR on sensory parameters and overall acceptability of biscuits

| Sensory parameters | | Control | 10 % GR | 15% GR | 20% GR | 25% GR |
|------------------------------|-----------------|----------|----------|----------|----------|----------|
| Appearance | Powdery surface | 3.5±1.11 | 2.2±1.87 | 2.0±1.87 | 2.0±1.87 | 2.0±2.28 |
| | Browning | 2.9±2.19 | 4.5±2.29 | 3.5±2.69 | 3.0±2.12 | 3.5±2.29 |
| Flavor | Raw | 0.2±0.45 | 0.2±0.45 | 1.0±1.73 | 0.2±0.71 | 0.5±0.71 |
| | Toasted | 4.6±2.07 | 5.2±1.79 | 4.5±2.29 | 4.3±2.59 | 3.5±2.18 |
| | Rancid | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| | Foreign | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| | Sweet | 5.7±1.30 | 5.0±1.87 | 5.4±1.08 | 4.9±1.81 | 4.4±1.47 |
| Texture | Crispness | 6.0±1.58 | 5.4±2.22 | 5.4±2.90 | 4.9±2.36 | 5.9±0.89 |
| | Hardness | 4.4±1.34 | 4.6±1.56 | 4.4±1.98 | 4.9±1.82 | 4.9±1.82 |
| | Coarseness | 3.5±1.50 | 3.7±1.79 | 4.8±1.79 | 4.5±2.29 | 4.5±2.29 |
| | Chewiness | 3.5±1.50 | 3.5±1.50 | 3.2±1.79 | 4.3±1.92 | 4.5±1.80 |
| | Adhesiveness | 2.7±1.72 | 2.8±1.68 | 2.3±1.57 | 3.1±1.43 | 3.4±1.29 |
| Overall acceptability | | 8.3±1.35 | 8.3±1.35 | 7.6±0.42 | 7.4±1.39 | 7.8±0.57 |

*Data are presented as Means ± S.D (n=5)

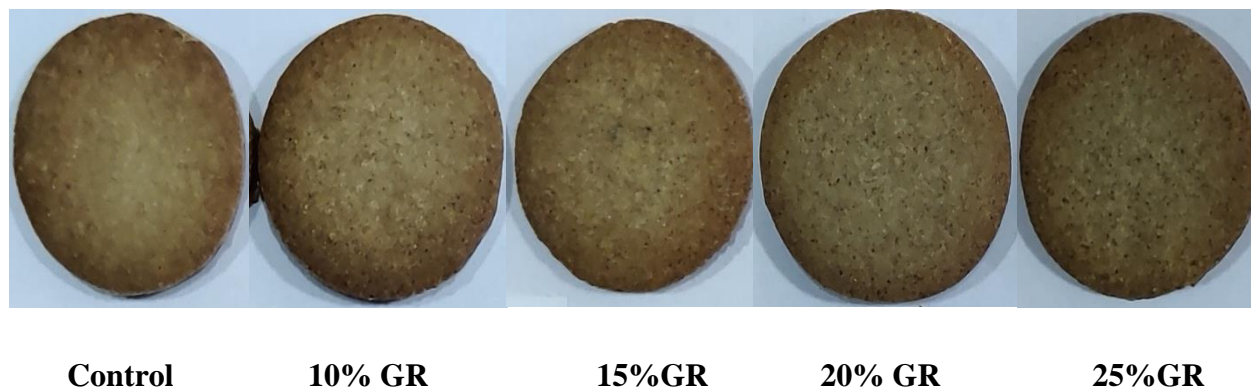


Fig 4.4: Images of biscuits prepared from different level of ghee residue and control

4.3.4 Physical & textural properties of biscuits

The spread ratio is one of the most important quality parameters for biscuits as it defines characteristics related to texture, chewiness, and overall mouth feel (Bose and Shams-ud-din, 2010). According to Florence *et al.* (2014), biscuits made from pearl millet flour have a low spread ratio. According to the result obtained based on the creep test of dough samples, the elasticity of the dough was reduced when GR was substituted and the elasticity of the biscuit decreased with the increase in the spread ratio of the biscuit. As shown in Table 4.9, the control had a spread ratio of 5.49, whereas the spread ratio increased to a maximum of 6.19 after the substitution of GR (25% GR).

Another parameter, the browning index, provides a brief idea about the extent of heat treatment. The colour of baked products is an important criterion for their preliminary acceptability. Additionally, the amount of browning determines the flavour of the final product (Mundt & Wedzicha, 2007). The Maillard reaction is primarily responsible for colour formation. Coloured compounds, such as hydroxy methyl furfural and melanoidins, accumulate during baking and are dependent on the compounds present in the products, the temperature, and the water activity of the system (Purlis, 2010). Baking time, moisture, dough composition, sugar, and protein content were all other considerations. The browning index was reduced with the substitution of GR ($p < 0.05$), which could be due to an increase in dough moisture content or a slight change in dough composition.

Further, the substitution of GR increased the cutting strength and hardness of the biscuits significantly ($p < 0.05$) and this could be attributed to the reduction in the fat content of biscuits that resulted in increased hardness. Chugh *et al.* (2013) also reported similar findings where the reduction in the fat levels increased the hardness of a composite biscuit.

Table 4.9: Effect of GR on physical & textural properties of pearl millet-based biscuit

| Physical property | Control | 10 % GR | 15% GR | 20% GR | 25% GR |
|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| Spread ratio | 5.49±0.38 ^a | 5.86±0.15 ^{ab} | 6.03±0.49 ^{ab} | 6.12±0.12 ^b | 6.19±0.08 ^b |
| Browning index | 101.26±1.0 ^b | 103.10±1.77 ^b | 101.52±0.60 ^b | 86.90±0.37 ^a | 86.48±0.50 ^a |
| Cutting strength (N) | 25.10 ±1.76 ^a | 28.44±0.98 ^b | 31.57 ±3.62 ^b | 32.46 ±4.41 ^b | 40.89±2.54 ^c |

*Data are presented as Means ± S.D (n=5)

^{ab} Means with different superscripts in different columns of a row differ significantly ($p < 0.05$)

4.3.5 The effect of dough resting time and levels of baking powder on the quality attributes of 20% GR-substituted biscuits

Table 4.10: Effect of dough resting time and different levels of baking powder on the proximate value of 20% GR biscuit

| Parameter | Baking powder (%) *Resting time (min) | 20 | 30 | 40 |
|---|--|---------------------------|---------------------------|--------------------------|
| Moisture (%) | 0.25 | 1.34±0.04 ^{aA} | 0.93±0.13 ^{aA} | 1.28±0.71 ^{aA} |
| | 0.5 | 1.1±0.11 ^{aA} | 2.08±0.56 ^{abB} | 1.84±0.48 ^{aA} |
| | 0.75 | 2.10±0.16 ^{abB} | 2.30±0.32 ^{abB} | 2.02±0.19 ^{aA} |
| Fat (%) | 0.25 | 18.51±0.11 ^{aA} | 19.43±0.35 ^{bA} | 19.64±0.11 ^{bB} |
| | 0.5 | 18.61±0.17 ^{aA} | 19.13±0.10 ^{bA} | 19.30±0.05 ^{bA} |
| | 0.75 | 19.38±0.09 ^{abB} | 19.50±0.10 ^{abA} | 19.62±0.03 ^{bB} |
| Acidity of extracted fat (% oleic acid) | 0.25 | 0.37±0.00 ^{bB} | 0.35±0.01 ^{aA} | 0.35±0.01 ^{aA} |
| | 0.5 | 0.35±0.01 ^{aA} | 0.36±0.01 ^{aA} | 0.35±0.01 ^{aA} |
| | 0.75 | 0.35±0.01 ^{aA} | 0.34±0.02 ^{aA} | 0.36±0.01 ^{aA} |
| Ash (%) | 0.25 | 1.22±0.03 ^{aA} | 1.26±0.02 ^{abA} | 1.29±0.01 ^{bA} |
| | 0.5 | 1.27±0.01 ^{bA} | 1.24±0.01 ^{aA} | 1.29±0.01 ^{bA} |
| | 0.75 | 1.40±0.02 ^{abB} | 1.41±0.06 ^{abB} | 1.34±0.02 ^{abB} |
| Acid insoluble ash (%) | 0.25 | 0.03±0.01 ^{aA} | 0.04±0.01 ^{aA} | 0.06±0.01 ^{bA} |
| | 0.5 | 0.04±0.01 ^{abB} | 0.05±0.01 ^{bB} | 0.09±0.01 ^{cB} |
| | 0.75 | 0.03±0.00 ^{aA} | 0.06±0.00 ^{bB} | 0.09±0.01 ^{cB} |
| Protein (%) | 0.25 | 5.87±0.10 ^{aA} | 6.72±0.13 ^{bA} | 6.62±0.17 ^{bA} |
| | 0.5 | 6.67±0.18 ^{abB} | 6.26±0.26 ^{aA} | 6.16±0.38 ^{aA} |
| | 0.75 | 6.67±0.13 ^{abB} | 6.73±0.02 ^{aA} | 6.71±0.06 ^{aA} |

Data are represented as mean±S.D (n=3)

^{abc} indicates within baking powder levels means with different superscripts are significantly different (P<0.05) from each other

^{ABC} indicates within dough resting time with different superscripts are significantly different (P<0.05) from each other

The dough resting time and baking powder levels are important factors, as the baking powder determines the texture and physical properties of a biscuit. It can be noted from the ANOVA Table 4.11, that moisture content differed non-significantly with the resting time ($p>0.05$) but significant with the baking powder levels ($p<0.05$). Fat content varied from 18.51 % to 19.64 % and it is worth noting that the resting time did not influence the ash content of the biscuit ($p>0.05$), however, the amount of baking powder significantly affected the ash content of the dough ($p<0.05$). Further, the resting time did not influence the protein composition of the biscuit ($p>0.05$), but the amount of baking powder had an impact which could be explained by the contribution of ammonium carbonate to the enhanced nitrogen content to the biscuit.

Table 4.11: p-values of main effects and interaction effects for proximate analysis

| Parameter | Moisture | Fat | Acidity of extracted fat | Ash | Acid insoluble ash | Protein |
|------------------------------------|---------------------|---------|--------------------------|---------------------|--------------------|---------------------|
| Baking powder | <0.001* | <0.001* | <0.220* | <0.001* | <0.001* | 0.002* |
| Resting time | 0.343 ^{NS} | <0.001* | 0.074 ^{NS} | 0.742 ^{NS} | <0.001* | 0.254 ^{NS} |
| Baking powder* Resting time | 0.048* | 0.001* | <0.016* | 0.001* | <0.001* | <0.001* |

p-value with superscript * and ^{NS} indicates that independent variables or its interaction variables or its interaction have a significant ($p<0.05$) & non-significant ($p>0.05$) effect on dependent variables respectively

4.3.6 The effect of dough resting time and baking powder levels on biscuit colour and water activity

Statistically, both colour and water activity were significantly different ($p<0.05$) with the variation in dough resting time and levels of baking powder. The lightness value increased with the increase in both doughs resting time and baking powder levels ($p<0.05$). The redness and yellowness, on the other hand, decreased ($p<0.05$). However, the water activity of the biscuits decreased as the resting time of the dough increased indicating that the dough had enough time with the ingredients to react and produce carbon dioxide. Baking reduced the moisture content of biscuits to nearly 2.0 % while, the water activity was in the range of 0.13-0.33.

Table 4.12: p-values of main effects and interaction effects for colour and water activity

| Parameter | L^* | a^* | b^* | a_w |
|--------------------------------|---------|---------|---------|---------|
| Baking powder | <0.001* | <0.001* | <0.001* | <0.001* |
| Resting time | <0.001* | <0.001* | <0.001* | <0.001* |
| Baking powder* Resting time | <0.001* | <0.001* | <0.001* | 0.001* |

p-value with superscript * indicates that independent variables or its interaction variables or its interaction have a significant ($p < 0.05$) effect on dependent variables respectively

Table 4.13: Effect of dough resting time and different levels of baking powder on the colour and water activity of 20% GR biscuit

| Parameters | Baking powder (%) | 20 | 30 | 40 |
|------------|---------------------|--------------------------|--------------------------|--------------------------|
| | *Resting time (min) | | | |
| L^* | 0.25 | 47.11±0.07 ^{aA} | 48.78±0.02 ^{bA} | 51.73±0.08 ^{cA} |
| | 0.5 | 50.92±0.06 ^{aB} | 53.69±0.16 ^{bB} | 55.20±0.31 ^{cB} |
| | 0.75 | 53.18±0.02 ^{aC} | 56.21±0.04 ^{bC} | 57.22±0.24 ^{cC} |
| a^* | 0.25 | 11.59±0.12 ^{cC} | 11.23±0.18 ^{bC} | 10.25±0.02 ^{aC} |
| | 0.5 | 10.53±0.07 ^{cB} | 9.51±0.03 ^{bB} | 8.19±0.03 ^{aB} |
| | 0.75 | 9.70±0.26 ^{bA} | 7.86±0.08 ^{aA} | 7.49±0.13 ^{aA} |
| b^* | 0.25 | 27.27±0.19 ^{bB} | 26.43±0.09 ^{aC} | 26.18±0.31 ^{aB} |
| | 0.5 | 26.20±0.13 ^{cA} | 25.77±0.17 ^{bB} | 24.40±0.46 ^{aA} |
| | 0.75 | 26.23±0.04 ^{cA} | 24.64±0.09 ^{bA} | 24.31±0.13 ^{aA} |
| a_w | 0.25 | 0.17±0.01 ^{bA} | 0.172±0.01 ^{bA} | 0.136±0.01 ^{aA} |
| | 0.5 | 0.328±0.01 ^{cC} | 0.224±0.16 ^{bB} | 0.147±0.01 ^{aA} |
| | 0.75 | 0.272±0.01 ^{aB} | 0.256±0.02 ^{aB} | 0.241±0.01 ^{aB} |

Data are represented as mean±S.D (n=3)

^{abc} indicates within baking powder levels means with different superscripts are significantly different ($P < 0.05$) from each other

^{ABC} indicates within dough resting time with different superscripts are significantly different ($P < 0.05$) from each other

4.3.7 Effect of dough resting time and different levels of baking powder on the sensory attributes

The interaction effect between dough resting time and levels of baking powder showed no significant difference in all parameters ($p < 0.05$). However, baking powder levels had a significant effect on the toasted and coarseness of the product ($p < 0.05$) which might be attributed to the leavening effect of baking powder thereby, resulting in coarseness in the product.

Table 4.14: Effect of dough resting time and different levels of baking powder on the sensory attributes

| Sensory Attribute | Baking powder (%) *Resting time (min) | 20 | 30 | 40 |
|-----------------------|--|-------------------------------|-------------------------------|--------------------------|
| Powdery Surface | 0.25 | 3.20±1.79 ^{aA} | 3.00±1.41 ^{aA} | 2.60±0.89 ^{aA} |
| | 0.5 | 3.30±2.17 ^{aA} | 3.10±1.75 ^{aA} | 3.30±1.64 ^{aA} |
| | 0.75 | 3.40±1.91 ^{aA} | 3.60±2.22 ^{aA} | 3.90±2.56 ^{aA} |
| Browning | 0.25 | 5.20±1.09 ^{aA} | 5.00±1.41 ^{aA} | 5.80±0.84 ^{aA} |
| | 0.5 | 3.80±2.16 ^{aA} | 4.80±2.28 ^{aA} | 5.60±1.67 ^{aA} |
| | 0.75 | 4.30±1.82 ^{aA} | 5.10±1.14 ^{aA} | 4.10±1.75 ^{aA} |
| Raw | 0.25 | 2.80±2.68 ^{aA} | 1.40±1.08 ^{aA} | 0.80±0.45 ^{aA} |
| | 0.5 | 2.00±2.00 ^{aA} | 1.80±1.48 ^{aA} | 0.80±0.84 ^{aA} |
| | 0.75 | 2.70±2.33 ^{aA} | 3.10±2.79 ^{aA} | 3.10±2.79 ^{aA} |
| Toasted | 0.25 | 3.00±1.87 ^{aA} | 2.60±1.52 ^{aA} | 3.00±1.87 ^{aA} |
| | 0.5 | 5.00±3.60 ^{aA} | 5.00±3.61 ^{aA} | 4.80±2.95 ^{aA} |
| | 0.75 | 3.10±2.01 ^{aA} | 2.80±1.79 ^{aA} | 2.70±1.64 ^{aA} |
| Rancid | 0.25 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 0.5 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 0.75 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Foreign flavour | 0.25 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 0.5 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 0.75 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Sweet | 0.25 | 4.80±0.47 ^{abA} | 4.40±0.82 ^{aA} | 5.50±0.50 ^{bA} |
| | 0.5 | 5.00±2.28 ^{aA} | 4.70±1.71 ^{aA} | 4.90±1.14 ^{aA} |
| | 0.75 | 4.30±1.30 ^{aA} | 4.00±1.87 ^{aA} | 4.00±1.87 ^{aA} |
| Crispness | 0.25 | 5.40±0.82 ^{aA} | 5.00±1.22 ^{aA} | 6.00±0.00 ^{aB} |
| | 0.5 | 5.80±2.28 ^{aA} | 5.80±1.09 ^{aA} | 5.80±0.45 ^{aAB} |
| | 0.75 | 5.20±0.76 ^{aA} | 5.70±0.84 ^{aA} | 5.40±0.42 ^{aA} |
| Hardness | 0.25 | 6.00±0.71 ^{aA} | 5.80±0.84 ^{aA} | 4.80±1.79 ^{aA} |
| | 0.5 | 4.40±2.41 ^{aA} | 5.00±2.74 ^{aA} | 4.40±2.79 ^{aA} |
| | 0.75 | 5.30±1.09 ^{aA} | 5.70±0.67 ^{aA} | 5.40±0.42 ^{aA} |
| Coarseness | 0.25 | 3.00±1.87 ^{aA} | 1.80±0.45 ^{aA} | 2.80±1.48 ^{aA} |
| | 0.5 | 5.30±2.54 ^{aA} | 5.30±2.59 ^{aA} | 5.60±2.79 ^{aA} |
| | 0.75 | 3.20±2.80 ^{aA} | 2.90±2.46 ^{aA} | 2.80±2.31 ^{aA} |
| chewiness | 0.25 | 2.90±1.34 ^{aA} | 2.10±0.55 ^{aA} | 3.10±1.88 ^{aA} |
| | 0.5 | 2.40±2.19 ^{aA} | 2.30±2.11 ^{aA} | 2.60±1.95 ^{aA} |
| | 0.75 | 2.62±1.19 ^{aA} | 2.60±1.08 ^{aA} | 2.00±1.73 ^{aA} |
| Adhesiveness | 0.25 | 2.80±1.09 ^{aA} | 2.60±0.89 ^{aA} | 3.40±1.78 ^{aA} |
| | 0.5 | 1.20±1.64 ^{aA} | 1.60±1.52 ^{aA} | 2.00±2.83 ^{aA} |
| | 0.75 | 2.00±1.73 ^{aA} | 2.00±1.73 ^{aA} | 2.00±1.73 ^{aA} |
| Overall acceptability | 0.25 | 6.80±0.84 ^{aA} | 6.60±1.14 ^{aA} | 7.00±0.71 ^{aA} |
| | 0.5 | 6.60±2.10 ^{aA} | 6.80±1.15 ^{aA} | 7.10±0.89 ^{aA} |
| | 0.75 | 7.70±1.09^{aA} | 7.20±0.91^{aA} | 6.90±1.14 ^{aA} |

Data are represented as mean±S.D (n=5)

^a indicates within baking powder levels mean with superscripts are significantly different ($P<0.05$)

^{AB} indicates within dough resting time with different superscripts are significantly different ($P<0.05$) from each other

Table 4.15: p-values of main effects and interaction effects for sensory attributes of GR substituted biscuit

| Parameter | Powdery Surface | Browning | Raw | Toasted | Sweet | Crispness | Hardness | Coarseness | Chewiness | Adhesiveness | Overall Acceptability |
|--|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|-----------------------|
| Baking powder | 0.595 ^{NS} | 0.368 ^{NS} | 0.113 ^{NS} | 0.039 [*] | 0.248 ^{NS} | 0.578 ^{NS} | 0.314 ^{NS} | 0.03 [*] | 0.865 ^{NS} | 0.112 ^{NS} | 0.485 ^{NS} |
| Resting time | 0.995 ^{NS} | 0.458 ^{NS} | 0.453 ^{NS} | 0.961 ^{NS} | 0.697 ^{NS} | 0.753 ^{NS} | 0.561 ^{NS} | 0.815 ^{NS} | 0.867 ^{NS} | 0.731 ^{NS} | 0.919 ^{NS} |
| Baking powder* Resting time | 0.975 ^{NS} | 0.549 ^{NS} | 0.714 ^{NS} | 0.999 ^{NS} | 0.910 ^{NS} | 0.685 ^{NS} | 0.948 ^{NS} | 0.968 ^{NS} | 0.859 ^{NS} | 0.975 ^{NS} | 0.777 ^{NS} |

p-value with superscript * and ^{NS} indicates that independent variables or its interaction variables or its interaction have a significant ($p < 0.05$) & non-significant ($p > 0.05$) effect on dependent variables respectively

4.3.8 Effect of dough resting time and different levels of baking powder on the spread ratio and hardness

It can be statistically interpreted that resting duration, baking powder levels, and resting time had no significant effect ($p>0.05$) on the hardness of biscuits (Table 4.17). However, the amount of baking powder in a biscuit has a major impact on its hardness. This could be due to the baking powder which aerates the product and turns it into a crisper and harder product. Biscuit spread ratio increased for the first 30 minutes of dough resting time, then dropped. However, with increase in the baking powder content, the spread ratio decreased ($p<0.05$).

Table 4.16: Effect of dough resting time and different levels of baking powder on the spread ratio and hardness of 20% GR biscuit

| | Baking powder (%) | 20 | 30 | 40 |
|----------------------|---------------------|--------------------------|--------------------------|--------------------------|
| | *Resting time (min) | | | |
| Spread ratio | 0.25 | 6.26±0.46 ^{ab} | 7.32±0.25 ^{bB} | 7.09±0.31 ^{bB} |
| | 0.5 | 6.25±0.07 ^{bB} | 6.28±0.27 ^{bA} | 5.76±0.35 ^{aA} |
| | 0.75 | 5.63±0.21 ^{aA} | 5.83±0.59 ^{aA} | 5.38±0.17 ^{aA} |
| Cutting strength (N) | 0.25 | 29.52±3.62 ^{aA} | 30.80±4.80 ^{aA} | 34.53±3.04 ^{aA} |
| | 0.5 | 31.78±4.70 ^{aA} | 32.07±4.70 ^{aA} | 35.90±6.08 ^{aA} |
| | 0.75 | 42.37±2.55 ^{ab} | 38.74±4.80 ^{aA} | 39.92±3.13 ^{aA} |

Data are represented as mean±S.D (n=5)

^{ab} indicates within baking powder levels mean with superscripts are significantly different ($P<0.05$)

^{AB} indicates within dough resting time with different superscripts are significantly different ($P<0.05$) from each other

Table 4.17: p-values of main effects and interaction effects for spread ratio and hardness

| Parameter | Spread ratio | Cutting strength |
|-----------------------------|--------------|----------------------|
| Baking powder | <0.001* | <0.001* |
| Resting time | <0.02* | <0.162 ^{NS} |
| Baking powder* Resting time | <0.001* | <0.360 ^{NS} |

p-value with superscript * and ^{NS} indicates that independent variables or its interaction variables or its interaction have a significant ($p<0.05$) & non-significant ($p>0.05$) effect on dependent variables respectively

4.4 Physical, textural, and rheological properties of GRP substituted to biscuit dough and the quality attributes of the resulting biscuit

4.4.1 Colour and water activity of dough

Results revealed that L^* (lightness value) declined as the levels of GRP were increased. WBD50 dough had the highest L^* value ($p < 0.05$), whereas GRP25 had the lowest ($p < 0.05$). Further, with the increase in the GRP substitution levels, a^* value was found to be increased however, the b^* value started decreasing ($p < 0.05$). With the substitution of GRP, the water activity of the WBD50 was recorded the value of 0.85. However, water activity increased which might be due to the water absorption capacity of ghee-residue powder.

Table 4.18: Effect on colour and water activity of biscuit dough upon substitution of GRP

| Dough sample | L^* | a^* | b^* | a_w |
|--------------|-------------------------|-------------------------|--------------------------|-------------------------|
| WBD50 | 45.44±0.34 ^e | -1.09±0.23 ^a | 19.22±0.29 ^a | 0.85±0.00 ^b |
| GRP10 | 42.67±0.34 ^d | 3.97±0.11 ^b | 22.25±0.16 ^d | 0.78±0.03 ^a |
| GRP15 | 40.35±0.02 ^c | 5.24±0.06 ^c | 21.69±0.19 ^{cd} | 0.82±0.02 ^{ab} |
| GRP20 | 39.14±0.03 ^b | 7.36±0.07 ^d | 21.15±0.29 ^c | 0.83±0.01 ^{ab} |
| GRP25 | 37.31±0.29 ^a | 8.29±0.06 ^e | 20.10±0.37 ^b | 0.85±0.01 ^b |

Data are represented as mean±S.D (n=3)

^{abcde} Means with a different superscript in different rows of a column differ significantly ($p < 0.05$)

Note: WBD50- Dough from RWF: PMF (50:50) with 100% shortening, GRP10-10% fat replaced with GRP, GRP15- 15% fat replaced with GRP, GRP20- 20% fat replaced with GRP, GRP25- 25% fat replaced with GRP

4.4.2 Textural properties of GRP substituted to dough

Generally, the strength of the dough is determined by its hardness and the dough should not be too firm, while making biscuits. Results revealed that the inclusion of GRP increased the dough's hardness substantially ($p < 0.05$). This might be due to the presence of protein in the GRP, as well as competence for the water which increased hardness. Further, Table 4.19 shows that the GRP25 dough was significantly harder as compared to any other dough ($p < 0.05$). The adhesiveness of the dough was found to be lower ($p < 0.05$) in the GRP10 dough sample however, it increased with an increase in the levels of substitution with GRP. GRP25 dough sample had a similar adhesiveness to that of the WBD50 dough sample. When compared to WBD50, the substitution of GRP reduced the springiness, chewiness, gumminess, and hardness. On the other hand, increased GRP levels further enhanced the values of springiness, chewiness, gumminess, resilience and hardness. However, statistically, the resilience values, among all the samples, did not differ significantly ($p > 0.05$). The GRP20 and GRP25, on the other hand, had a slightly greater resilience value.

Table 4.19: Effect of GRP substitution on Textural properties of biscuit dough

| Dough sample | Hardness (N) | Adhesiveness | Springiness | Cohesiveness | Gumminess | Chewiness | Resilience |
|--------------|--------------------------|-------------------------------|------------------------|------------------------|-------------------------|-------------------------|------------------------|
| WBD50 | 17.56±2.29 ^a | -1069.14±46.33 ^b | 0.24±0.03 ^b | 0.24±0.03 ^b | 4.18±0.10 ^a | 1.01±0.12 ^b | 0.02±0.00 ^a |
| GRP10 | 107.60±2.47 ^b | -2163.32±94.95 ^a | 0.05±0.01 ^a | 0.10±0.01 ^a | 11.61±0.52 ^b | 0.79±0.10 ^{ab} | 0.02±0.00 ^a |
| GRP15 | 133.80±1.35 ^c | -1532.02±237.67 ^{ab} | 0.06±0.01 ^a | 0.10±0.01 ^a | 14.03±1.25 ^c | 0.70±0.09 ^a | 0.02±0.00 ^a |
| GRP20 | 134.36±2.27 ^c | -1457.98±457.50 ^b | 0.06±0.01 ^a | 0.11±0.00 ^a | 14.94±0.59 ^c | 0.88±0.10 ^{ab} | 0.03±0.00 ^b |
| GRP25 | 153.43±1.43 ^d | -1388.23±465.68 ^{ab} | 0.06±0.01 ^a | 0.11±0.01 ^a | 17.36±0.75 ^d | 1.00±0.15 ^{ab} | 0.03±0.00 ^b |

Data are represented as mean±S.D (n=5)

^{abcd} Means with a different superscript in different rows of a columns differ significantly (p<0.05)



10% GRP



15% GRP



20% GRP



25% GRP

Fig 4.5: Images of biscuit prepared from different levels of ghee residue powder

4.4.3 Rheological properties of GRP substituted dough

4.4.3.1 Creep test of a GRP substituted dough

All of the samples exhibited viscoelastic behaviour when constant stress was applied; the strain was increasing and partial reformation occurred when the load was removed during the recovery period. It was concluded by Zhang *et al.* (2017) that maximal deformation during the creep test can be directly correlated with the dough strength. Also, deformation resistance was shown to be higher in dough samples with a stronger dough structure (Wang & Sun, 2002). According to Manohar & Rao (2002), the highest per cent recovery was attained either due to high protein content or longer mixing time. Maximum strain indicates dough extensibility, and as shown in Figure 4.6, all of the dough samples showed an upsurge in strain and partial reformation after the applied stress was withdrawn (similar results were reported by Abebe *et al.*, 2015). Only the GRP10 dough sample had the lowest strain, whereas the rest were similar to WBD50. GRP10 dough has a lower maximum strain and it was less extensible. This could be directly related to a biscuit's spread ratio. Also, GRP10 was less extensible in nature, hence, the spread ratio was greater. The elasticity of the dough was related to the highest recovery and percentage recovery. All the dough samples had a higher percentage recovery except for control (WBD50). The spread ratio of the biscuits proved that the dough was elastic in nature, and the textural examination using TPA of the dough confirmed that the dough was elastic in nature as well.

Table 4.20: Effect of GRP substitution on rheological characteristics of biscuit dough

| Dough sample | Max. Strain | Recovery | Percentage Recovery |
|--------------|-------------------------|------------|---------------------|
| WBD50 | 0.03±0.01 ^{ab} | 0.010±0.00 | 09.40±4.40 |
| GRP10 | 0.01±0.01 ^a | 0.014±0.01 | 39.54±4.49 |
| GRP15 | 0.04±0.00 ^b | 0.006±0.00 | 15.74±4.9 |
| GRP20 | 0.03±0.01 ^{ab} | 0.009±0.00 | 20.82±6.57 |
| GRP25 | 0.03±0.02 ^{ab} | 0.012±0.00 | 20.28±6.4 |

* Data are represented as mean±S.D (n=3)

^{ab} Means with different superscript in different rows of a column differ significantly (p<0.05)

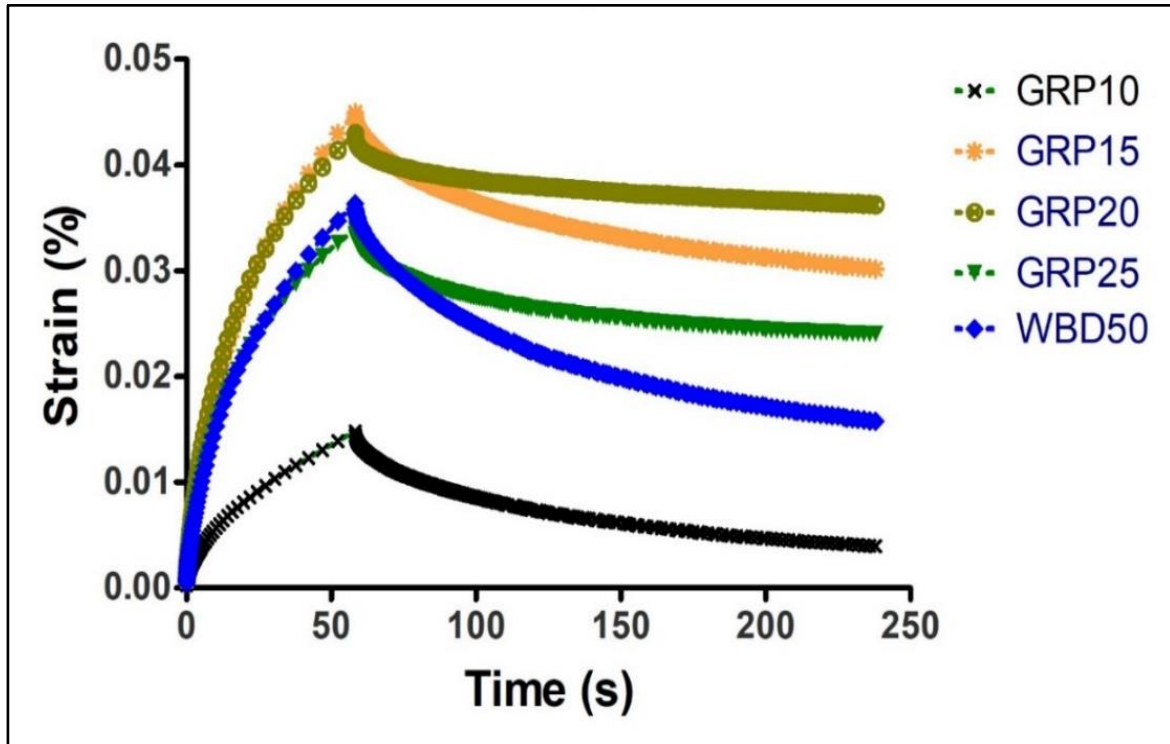


Fig 4.6: Creep recovery of GRP substituted dough

4.4.3.2 Frequency sweep of GRP substituted dough samples

The dough's viscoelastic characteristics can be determined through oscillatory measurements. The test parameters, storage modulus (G') and loss modulus (G'') versus angular frequency were utilized to examine the stiffness and elastic versus viscous behaviour of the dough under test conditions, as shown in Figure 4.7. Storage modulus (G') represents the dough's solid or elastic behaviour, whereas loss modulus (G'') represents the dough's liquid or viscous behaviour for all samples, an increase in dynamic moduli values with increasing oscillation frequency within the range of 0.1 to 100 Hz can be observed. The storage modulus for all the dough samples was higher than the loss modulus suggesting that the all-dough samples were solid and showed elastoviscous behaviour (figure 4.7). The storage modulus of ghee-residue powder substituted dough samples were significantly lower than the control (WBD50) and also with the substitution of ghee-residue powder, the loss moduli was decreasing.

$\tan \delta$ is a ratio of loss modulus to the storage modulus. It gives an idea about the viscous or elastic behaviour of a sample. All the dough samples were showing lower loss modulus due to the higher values of storage modulus which signifies that the dough samples were elastic in nature. The low value of $\tan \delta$ indicates a more elastic dough (Puppo *et al.*, 2005).

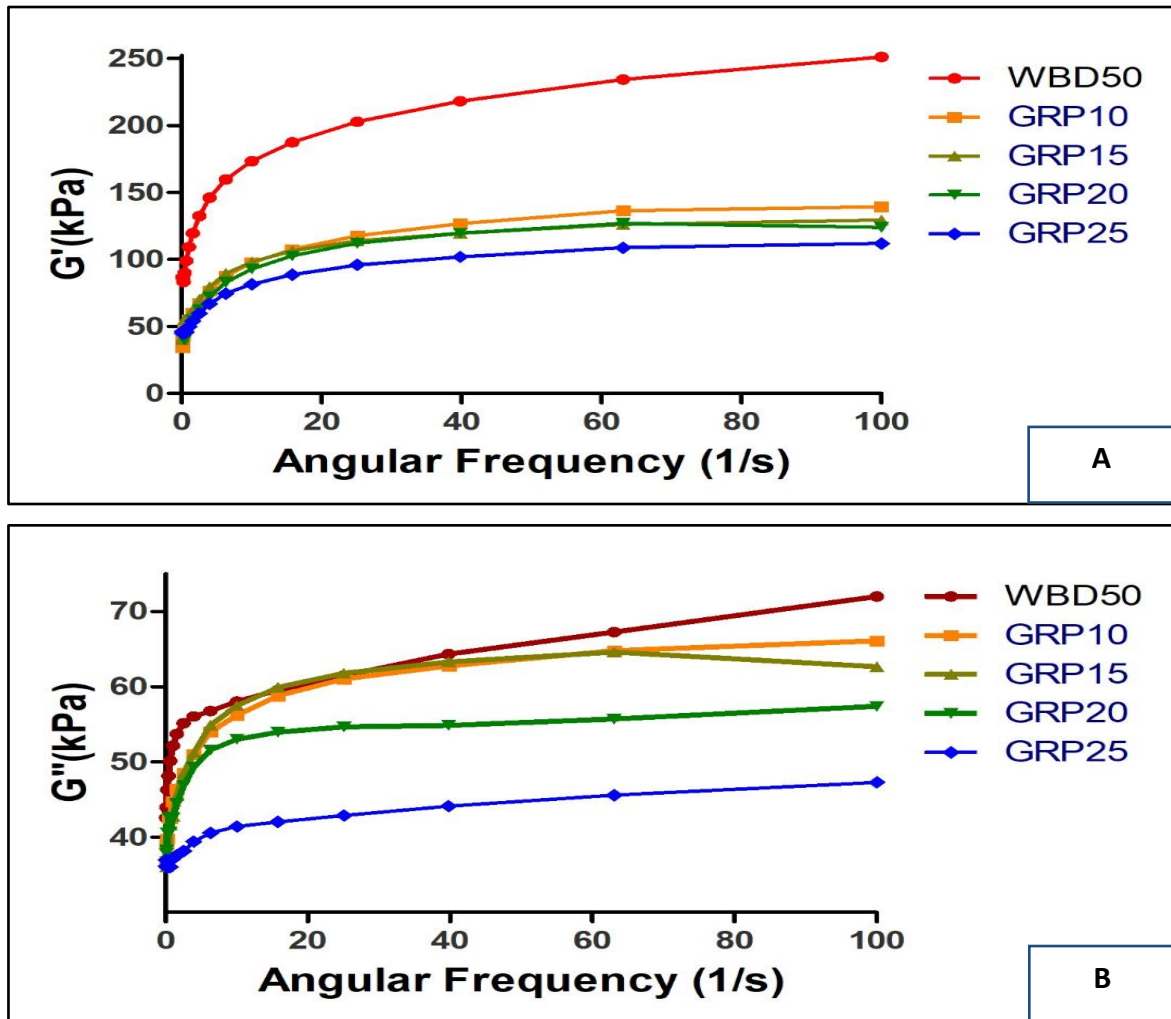


Fig 4.7: Frequency sweep of GRP substituted dough samples (A) Storage modulus, (B) Loss modulus

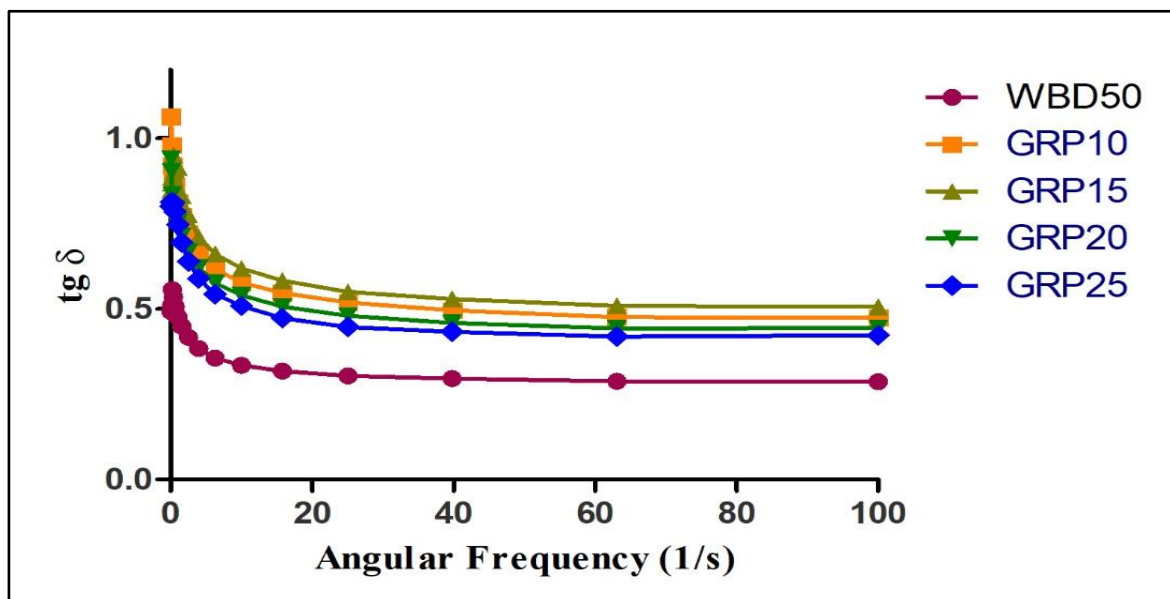


Fig 4.8: Dynamic viscoelastic loss factor with frequency dependence ($\tan \delta$) for ghee-residue powder substituted doughs

4.4.4 The effect of GRP on the quality parameters of biscuits

4.4.4.1 Effect of different levels of GRP substitution on the proximate values of pearl millet-based biscuit

The chemical composition of the ghee-residue powder substituted at different levels in biscuits is shown in Table 4.21. The moisture content of the samples ranged from 1.44 % to 2.49 %, which was sufficient for storing the product as well as achieving the desired textural features of a biscuit. Due to the water holding capacity of proteins, the moisture content of these biscuits was higher than the biscuits containing GRP (Gallagher *et al.*, 2005). Thalor (2021) found that the GRP had a water binding capacity of ~2.2 g/g protein. However, the type of soluble proteins present in the sample determines the water binding capability. The fat content of the samples increased when the ghee-residue powder was substituted because the ghee-residue powder contains high amounts of fat. Fat content ranged from 17.34 % to 23.44 %. The extracted fat's acidity ranged from 0.38 % to 0.28 % oleic acid. The biscuit's protein composition ranged from 6.72 % to 11.30 %. Due to the high protein content of ghee-residue powder, the protein content of biscuits was increased. With the substitution of GRP, the ash content and acid insoluble ash were also increased.

Table 4.21: Effect of different levels of GRP on the proximate values of pearl millet-based biscuit

| Treatments | Moisture (% Wet Basis) | Fat (%) | Acidity of extracted fat (% oleic acid) | Protein (%) | Ash (%) | Acid insoluble ash (%) |
|------------|------------------------|-------------------------|---|-------------------------|------------------------|------------------------|
| 10 % GRP | 1.65±0.04 ^b | 17.34±0.12 ^a | 0.38±0.01 ^c | 6.72±0.03 ^a | 1.03±0.01 ^a | 0.03±0.00 ^a |
| 15% GRP | 1.74±0.01 ^b | 19.67±0.01 ^b | 0.36±0.01 ^c | 8.08±0.01 ^b | 1.12±0.01 ^b | 0.04±0.00 ^a |
| 20% GRP | 2.49±0.05 ^c | 21.44±0.09 ^c | 0.33±0.01 ^b | 10.23±0.09 ^c | 1.18±0.01 ^c | 0.04±0.01 ^a |
| 25% GRP | 1.44±0.05 ^a | 23.44±0.06 ^d | 0.28±0.03 ^a | 11.30±0.05 ^d | 1.23±0.01 ^d | 0.05±0.0 ^b |

* Data are represented as mean±S.D (n=3)

^{abcd} Means with different superscript in different rows of a column differ significantly (p<0.05)

Note: 10 %GRP- 10 % fat replaced with GRP, 15 %GRP- 15 % fat replaced with GRP. 20 % GRP- 20 % fat replaced with GRP & 25 % GRP- 25 % fat replaced with GRP.

4.4.4.2 Effect of different levels of GRP substitution on the colour and water activity of pearl millet-based biscuit

With the different levels of fat substitution with GRP, the color values and lightness were influenced. This effect was due to the interaction between protein and sugar in the baking process, which leads to the Maillard reaction and also caramelization. The lightness and yellowness values were decreasing, while the redness value was increasing. Gallagher *et al.* (2005) reported that with the increase in the dairy protein powders, the lightness of the product was decreased. The brightness ranged from 54.39 to 48.39, the redness from 6.24 to 10.48, and the yellowness from 27.72 to 25.82 for GRP substituted biscuits. The biscuit's water activity ranged from 0.23 to 0.27. When compared to the GR-substituted biscuits, these biscuits had a higher water activity. The water activity was primarily attributable to the biscuit's lower spread ratio.

Table 4.22: Effect on colour and water activity of Biscuit upon replacing fat with GRP

| Treatments | L* | a* | b* | a _w |
|------------|-------------------------|-------------------------|--------------------------|-------------------------|
| 10 % GRP | 54.39±0.03 ^c | 6.24±0.03 ^a | 26.79±0.08 ^{ab} | 0.237±0.03 ^a |
| 15% GRP | 49.38±0.35 ^b | 8.46±0.07 ^b | 27.72±0.95 ^b | 0.276±0.01 ^b |
| 20% GRP | 48.60±0.27 ^a | 10.91±0.12 ^c | 25.95±0.32 ^a | 0.273±0.01 ^b |
| 25% GRP | 48.39±0.28 ^a | 10.48±0.07 ^c | 25.82±0.05 ^a | 0.271±0.00 ^b |

* Data are represented as mean±S.D (n=3)

^{abc} Means with different superscript in different rows of a column differ significantly (p<0.05)

4.4.4.3 Physical & textural properties of GRP substituted biscuit

Table 4.23 shows the spread ratio, cutting strength, and browning index of ghee-residue powder substituted biscuits. As the levels of substitution of fat with ghee-residue powder increased, the spread ratio of the biscuits decreased. It was evident from the rheological data obtained that the biscuit doughs were elastic in nature, as an increase in protein content leads the dough to shrink as it recovers from deformation. As a result, the spread ratio shrunk. Sanchez *et al.* (1989) found that increasing the content of whey protein concentrate reduced the spread ratio.

The hardness of a biscuit was determined by its cutting strength. The hardness increased significantly (p<0.05) when the amount of ghee-residue powder increased with substitution. According to Giese (1994), the milk casein protein produces hardness in biscuits as the levels of casein rise. The highest cutting strength was recorded for the 25% GRP biscuit.

Table 4.23: Physical properties of GRP substituted biscuit

| Physical property | 10 % GRP | 15% GRP | 20% GRP | 25% GRP |
|----------------------|--------------------------|--------------------------|-------------------------|-------------------------|
| Spread ratio | 5.31±0.29 ^b | 4.82±0.17 ^a | 4.64±0.28 ^a | 4.49±0.08 ^a |
| Cutting strength (N) | 55.40 ±6.17 ^a | 58.35 ±3.53 ^a | 73.94±4.60 ^b | 84.24±7.55 ^c |
| Browning index | 79.69±0.27 ^a | 99.41±4.2 ^c | 96.27±2.07 ^b | 92.67±0.57 ^b |

* Data are represented as mean±S.D (n=5)

^{abc} Means with different superscript in different columns of a row differ significantly (p<0.05)

Surface colour is a key quality element in bakery products, as it is linked to odour, taste, and aesthetic characteristics that are important for consumers. Browning is defined as the production of a characteristic colour, such as yellow, gold, or brown, depending on the substance (*i.e.*, ingredients, operating conditions and product specifications). Browning in bakery products is a dynamic process influenced mostly by the system's temperature and water activity, and resulting from the synthesis and build-up of coloured chemicals during baking (Purlis, 2010). The maximum browning index was obtained for 15 % GRP biscuit and the lowest for 10 % GRP (Table 4.3).

4.4.4.4 Sensory analysis of fat substituted GRP-pearl millet-based biscuit

In comparison to the control, the powdery surface was reduced with the levels of substitution of ghee-residue powder. The browning score did not differ much from the sensory score point of view. With GRP incorporation, the raw flavor was reduced. Replacing fat with GRP, on the other hand, improved the toasted flavor. The sweetness of the biscuits did not differ significantly (p<0.05). There was no significant (p<0.05) variation in textural qualities such as crispness, coarseness, hardness, chewiness, and adhesiveness. Finally, the overall acceptability of the 25% GRP biscuit received a maximum score.

4.4.5 The effect of dough resting time and levels of baking powder on the different quality attributes of 25 % GRP biscuits

4.4.5.1 The effect of dough resting time and levels of baking powder on the quality attributes of 25 % GRP biscuit

The 25% GRP biscuit was selected from the sensory evaluation to analyse the interaction effect of baking powder levels and dough resting time. From Table 4.25, baking powder levels have no significant effect (p<0.05) on the acidity of the extracted fat and acid insoluble ash. Both baking powder level and resting time interaction did not affect the protein

Table 4.24: Effect of substituting fat with GRP on sensory parameters and overall acceptability of biscuit

| Sensory parameters | | 10 % GRP | 15% GRP | 20% GRP | 25% GRP |
|------------------------------|-----------------|-------------------------|------------------------|-------------------------|------------------------|
| Appearance | Powdery surface | 3.50±1.65 | 3.00±2.00 | 3.00±2.00 | 3.00±2.00 |
| | Browning | 5.00±0.00 | 4.80±0.45 | 4.50±0.87 | 5.00±0.00 |
| Flavor | Raw | 2.50±2.29 | 2.50±2.00 | 2.20±1.89 | 1.80±1.95 |
| | Toasted | 3.90±1.14 | 4.20±0.90 | 4.90±1.24 | 5.00±1.06 |
| | Rancid | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | Foreign flavor | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | Sweet | 4.90±0.22 | 4.90±0.22 | 4.80±0.27 | 4.50±0.50 |
| Texture | Crispness | 6.00±1.58 | 5.80±1.48 | 5.70±1.30 | 5.80±1.30 |
| | Hardness | 5.80±1.48 | 5.50±1.50 | 5.70±1.09 | 5.80±0.83 |
| | Coarseness | 3.70±0.97 | 4.00±0.6 | 3.90±0.74 | 3.70±0.84 |
| | Chewiness | 5.00±1.41 | 4.80±1.09 | 4.80±1.10 | 4.90±1.43 |
| | Adhesiveness | 3.90±1.67 | 3.97±1.67 | 4.20±1.44 | 4.20±1.44 |
| Overall acceptability | | 8.25±0.43 ^{ab} | 7.50±0.35 ^a | 8.12±0.54 ^{ab} | 8.50±0.61 ^b |

Data are represented as mean±S.D (n=5)

^{ab} Means with different superscript in different columns of a row differ significantly (p<0.05)

Table 4.25: p-values of main effects and interaction effects for proximate analysis of 25% GRP biscuit

| Parameter | Moisture | Fat | Acidity of extracted fat | Ash | Acid insoluble ash | Protein |
|--------------------------------|----------|---------|--------------------------|---------|---------------------|---------------------|
| Baking powder | <0.001* | <0.001* | <0.220 ^{NS} | <0.001* | 0.588 ^{NS} | <0.001* |
| Resting time | <0.001* | <0.001* | <0.001* | 0.016* | 0.04* | 0.017* |
| Baking powder* Resting time | <0.001* | 0.001* | 0.007* | 0.001* | 0.005* | 0.346 ^{NS} |

p-value with superscript * and ^{NS} indicates that independent variables or its interaction variables or its interaction have a significant (p<0.05) & non-significant (p>0.05) effect on dependent variables respectively

content. As the resting time and baking powder levels increased, the moisture content increased. Maximum fat content was observed when the baking powder level was 0.5 %.

Table 4.26: Effect of dough resting time and different levels of baking powder on the proximate value of 25% GRP biscuit

| Parameters | Baking powder (%) | 20 | 30 | 40 |
|---|---------------------|--------------------------|---------------------------|--------------------------|
| | *Resting time (min) | | | |
| Moisture (%) | 0.25 | 1.12±0.15 ^{abA} | 1.02±0.03 ^{aA} | 1.34±0.07 ^{cA} |
| | 0.5 | 1.28±0.08 ^{aA} | 1.44±0.05 ^{aB} | 1.82±0.09 ^{bB} |
| | 0.75 | 2.71±0.08 ^{cB} | 2.02±0.12 ^{aC} | 2.40±0.08 ^{bC} |
| Fat (%) | 0.25 | 19.61±0.40 ^{bA} | 18.67±0.24 ^{aB} | 20.22±0.91 ^{bA} |
| | 0.5 | 22.08±0.29 ^{bB} | 23.31±0.06 ^{aC} | 23.15±0.07 ^{aC} |
| | 0.75 | 19.55±0.34 ^{bA} | 17.21±0.42 ^{aA} | 21.95±0.31 ^{cB} |
| Acidity of extracted fat (% oleic acid) | 0.25 | 0.35±0.01 ^{abB} | 0.35±0.01 ^{bB} | 0.33±0.02 ^{aB} |
| | 0.5 | 0.30±0.01 ^{bA} | 0.28±0.01 ^{abA} | 0.28±0.01 ^{aA} |
| | 0.75 | 0.34±0.01 ^{bB} | 0.36±0.01 ^{cB} | 0.31±0.01 ^{aB} |
| Ash (%) | 0.25 | 1.03±0.12 ^{aAB} | 1.03±0.08 ^{aA} | 0.79±0.17 ^{aA} |
| | 0.5 | 0.84±0.03 ^{aA} | 1.23±0.01 ^{bB} | 1.29±0.01 ^{bB} |
| | 0.75 | 1.16±0.07 ^{aB} | 1.14±0.06 ^{aAB} | 1.23±0.04 ^{aB} |
| Acid insoluble ash (%) | 0.25 | 0.09±0.01 ^{bB} | 0.06±0.01 ^{abAB} | 0.04±0.02 ^{aA} |
| | 0.5 | 0.06±0.01 ^{bA} | 0.05±0.01 ^{aA} | 0.06±0.00 ^{bA} |
| | 0.75 | 0.07±0.01 ^{aA} | 0.06±0.00 ^{aC} | 0.06±0.01 ^{aA} |
| Protein (%) | 0.25 | 10.54±0.29 ^{aA} | 10.62±0.19 ^{aA} | 10.88±0.81 ^{aA} |
| | 0.5 | 11.59±0.16 ^{aB} | 11.31±0.05 ^{aB} | 11.61±0.17 ^{aB} |
| | 0.75 | 11.20±0.17 ^{aB} | 11.31±0.27 ^{aB} | 11.49±0.07 ^{aB} |

Data are represented as mean±S.D (n=3)

^{abc} indicates within baking powder levels means with different superscripts are significantly different ($P<0.05$) from each other

^{ABC} indicates within dough resting time with different superscripts are significantly different ($P<0.05$) from each other

4.4.5.2 The effect of dough resting time and baking powder levels on biscuit colour and water activity of 25 %GRP biscuits

Both the colour and the water activity were significantly different ($p < 0.05$) as per ANOVA Table 4.28, except for the redness, which was non-significant at the baking powder levels.

Table 4.27: Effect of dough resting time and different levels of baking powder on the colour and water activity of 25% GRP biscuit

| Parameters | Baking powder (%) *Resting time (min) | 20 | 30 | 40 |
|----------------|--|--------------------------|--------------------------|---------------------------|
| L* | 0.25 | 49.11±0.06 ^{bC} | 52.33±0.20 ^{cC} | 48.36±0.02 ^{aB} |
| | 0.5 | 44.19±0.19 ^{aA} | 49.38±0.35 ^{cB} | 46.71±0.38 ^{bA} |
| | 0.75 | 48.74±0.04 ^{bB} | 47.97±0.27 ^{aA} | 47.75±0.27 ^{aB} |
| a* | 0.25 | 11.69±0.02 ^{cB} | 10.32±0.21 ^{aA} | 11.14±0.05 ^{bA} |
| | 0.5 | 11.97±0.07 ^{cC} | 10.48±0.07 ^{aA} | 10.94±0.14 ^{bA} |
| | 0.75 | 10.92±0.04 ^{aA} | 11.19±0.08 ^{bB} | 10.95±0.06 ^{aA} |
| b* | 0.25 | 27.64±0.11 ^{bB} | 26.68±0.04 ^{aC} | 26.38±0.26 ^{aB} |
| | 0.5 | 26.04±0.09 ^{aA} | 25.82±0.05 ^{aA} | 25.89±0.35 ^{aAB} |
| | 0.75 | 26.12±0.33 ^{bA} | 26.31±0.16 ^{bB} | 25.48±0.16 ^{aA} |
| a _w | 0.25 | 0.22±0.01 ^{aB} | 0.26±0.01 ^{cB} | 0.23±0.01 ^{bB} |
| | 0.5 | 0.17±0.02 ^{aA} | 0.27±0.01 ^{cB} | 0.21±0.01 ^{bA} |
| | 0.75 | 0.24±0.01 ^{bB} | 0.17±0.01 ^{aA} | 0.26±0.01 ^{aC} |

Data are represented as mean±S.D (n=3)

^{abc} indicates within baking powder levels means with different superscripts are significantly different ($P < 0.05$) from each other

^{ABC} indicates within dough resting time with different superscripts are significantly different ($P < 0.05$) from each other

4.4.5.3 Effect of dough resting time and different levels of baking powder on the sensory attributes of 25 % GRP biscuits

All the factors did not exhibit a significant difference ($p < 0.05$) in dough resting time and baking powder levels. The levels of baking powder added have shown a substantial ($p < 0.05$) impact on a product's powdery surface, chewiness, adhesiveness, and coarseness. The

hardness, toasted, and sweet flavour of a product are affected by the interaction impact of dough resting time and baking powder content ($p < 0.05$).

Table 4.28: *p*-values of main effects and interaction effects for colour and water activity

| Parameter | L* | a* | B* | a _w |
|-----------------------------|---------|---------------------|---------|----------------|
| Baking powder | <0.001* | 0.082 ^{NS} | <0.001* | <0.001* |
| Resting time | <0.001* | <0.001* | <0.001* | <0.001* |
| Baking powder* Resting time | <0.001* | <0.001* | <0.001* | <0.001* |

p-value with superscript * and ^{NS} indicates that independent variables or its interaction variables or its interaction have a significant ($p < 0.05$) & non-significant ($p > 0.05$) effect on dependent variables respectively

Table 4.29: Effect of dough resting time and different levels of baking powder on the sensory attributes

| Sensory attribute | Baking powder (%) | 20 | 30 | 40 |
|-------------------|---------------------|--------------------------|-------------------------|-------------------------|
| | *Resting time (min) | | | |
| Powdery surface | 0.25 | 1.60±0.42 ^{aA} | 1.60±0.42 ^{aA} | 2.10±0.42 ^{aA} |
| | 0.5 | 2.30±0.57 ^{aAB} | 3.00±2.00 ^{aA} | 2.50±0.35 ^{aA} |
| | 0.75 | 2.50±0.35 ^{aB} | 2.70±0.57 ^{aA} | 2.50±0.35 ^{aA} |
| Browning | 0.25 | 4.10±0.74 ^{aA} | 4.20±0.57 ^{aA} | 4.50±0.50 ^{aA} |
| | 0.5 | 4.20±0.91 ^{aA} | 5.00±0.00 ^{aA} | 4.50±0.50 ^{aA} |
| | 0.75 | 3.80±0.57 ^{aA} | 4.50±0.61 ^{aA} | 4.20±0.57 ^{aA} |
| Raw | 0.25 | 0.80±0.27 ^{aA} | 2.40±0.65 ^{bA} | 2.00±0.79 ^{bA} |
| | 0.5 | 1.90±0.42 ^{aAB} | 1.80±1.96 ^{aA} | 2.30±0.57 ^{aA} |
| | 0.75 | 2.80±1.03 ^{aB} | 2.30±0.57 ^{aA} | 2.30±0.90 ^{aA} |
| Toasted | 0.25 | 4.00±0.79 ^{bA} | 4.20±0.57 ^{bA} | 2.80±0.57 ^{aA} |
| | 0.5 | 3.40±0.42 ^{aA} | 5.00±1.06 ^{aA} | 4.60±0.82 ^{aB} |
| | 0.75 | 4.40±0.96 ^{aA} | 4.40±0.65 ^{aA} | 4.50±1.00 ^{aB} |
| Rancid | 0.25 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 0.5 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 0.75 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |

| | | | | |
|------------------------------|------|--------------------------|--------------------------|--------------------------|
| Foreign flavour | 0.25 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 0.5 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 0.75 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Sweet | 0.25 | 4.90±0.74 ^{aA} | 5.40±0.42 ^{aB} | 5.60±0.65 ^{aA} |
| | 0.5 | 5.70±0.57 ^{aA} | 4.50±0.50 ^{aA} | 4.90±0.65 ^{aA} |
| | 0.75 | 4.90±0.65 ^{aA} | 5.31±0.57 ^{aAB} | 5.20±0.45 ^{aA} |
| Crispness | 0.25 | 7.00±0.79 ^{bB} | 5.30±0.97 ^{aA} | 6.10±0.42 ^{abA} |
| | 0.5 | 6.00±0.61 ^{aAB} | 5.80±1.30 ^{aA} | 5.60±0.65 ^{aA} |
| | 0.75 | 5.70±0.76 ^{aA} | 5.50±0.79 ^{aA} | 5.70±0.76 ^{aA} |
| Hardness | 0.25 | 7.20±0.76 ^{bB} | 5.40±0.65 ^{aA} | 6.00±0.61 ^{aA} |
| | 0.5 | 5.90±0.65 ^{aA} | 5.80±1.30 ^{aA} | 5.60±0.65 ^{aA} |
| | 0.75 | 5.30±0.76 ^{aA} | 5.30±0.76 ^{aA} | 5.30±0.76 ^{aA} |
| Coarseness | 0.25 | 4.90±0.42 ^{aA} | 4.40±0.96 ^{aA} | 4.60±0.42 ^{aA} |
| | 0.5 | 4.10±0.42 ^{aA} | 3.70±0.84 ^{aA} | 3.90±0.63 ^{aA} |
| | 0.75 | 4.60±1.19 ^{aA} | 4.00±0.79 ^{aA} | 4.60±0.65 ^{aA} |
| chewiness | 0.25 | 4.50±0.50 ^{aA} | 6.20±0.57 ^{bB} | 5.40±0.65 ^{abB} |
| | 0.5 | 4.70±0.57 ^{aA} | 4.90±1.43 ^{aAB} | 4.00±0.79 ^{aA} |
| | 0.75 | 4.60±0.65 ^{aA} | 4.40±0.65 ^{aA} | 4.60±0.65 ^{aAB} |
| Adhesiveness | 0.25 | 3.60±0.65 ^{aA} | 5.00±0.79 ^{bB} | 4.80±0.27 ^{bB} |
| | 0.5 | 4.20±0.57 ^{aA} | 4.20±1.44 ^{aAB} | 4.40±0.65 ^{aA} |
| | 0.75 | 3.30±0.90 ^{aA} | 3.00±0.79 ^{aA} | 3.30±0.90 ^{aAB} |
| Overall acceptability | 0.25 | 7.40±0.42 ^{aA} | 8.10±0.42 ^{aA} | 7.80±0.57 ^{aA} |
| | 0.5 | 8.00±0.35 ^{aAB} | 8.50±0.61 ^{aA} | 8.00±0.35 ^{aA} |
| | 0.75 | 8.10±0.41 ^{aB} | 8.00±0.35 ^{aA} | 8.10±0.41 ^{aA} |

Data are represented as mean±S.D (n=5)

^a indicates within baking powder levels mean with superscripts are significantly different (P<0.05)

^{AB} indicates within dough resting time with different superscripts are significantly different (P<0.05) from each other

Table 4.30: p-values of main effects and interaction effects for sensory attributes of 25% GRP biscuit

| Parameter | Powdery surface | Browning | Raw | Toasted | sweet | crispness | Hardness | Coarseness | chewiness | Adhesiveness | Overall acceptability |
|--|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|-----------------------|
| Baking powder | 0.009* | 0.178 ^{NS} | 0.103 ^{NS} | 0.023 ^{NS} | 0.463 ^{NS} | 0.247 ^{NS} | 0.004 ^{NS} | 0.056* | 0.006* | <0.001* | 0.048* |
| Resting time | 0.552 ^{NS} | 3.056 ^{NS} | 0.492 ^{NS} | 0.078 ^{NS} | 0.748 ^{NS} | 0.074 ^{NS} | 0.062 ^{NS} | 0.249 ^{NS} | 0.100 ^{NS} | 0.283 ^{NS} | 0.087 ^{NS} |
| Baking powder* Resting time | 0.653 ^{NS} | 0.568 ^{NS} | 0.098 ^{NS} | 0.012* | 0.011* | 0.222 ^{NS} | 0.031* | 0.979 ^{NS} | 0.037* | 0.202 ^{NS} | 0.270 ^{NS} |

p-value with superscript * and ^{NS} indicates that independent variables or its interaction variables or its interaction have a significant ($p < 0.05$) & non-significant ($p > 0.05$) effect on dependent variables respectively

4.4.5.4 Effect of dough resting time and different levels of baking powder on the spread ratio and hardness of 25 %GRP biscuits

Table 4.32 shows that there was no interaction effect between baking powder level and dough resting time on the spread ratio of the biscuits. However, as the baking powder level increased, the spread ratio was also increasing. Dough resting time did not affect the spread ratio. Hardness/cutting strength was significantly affected by the dough resting time, baking powder and their respective interactions. An increase in the level of baking powder decreased the hardness of a biscuit, while with the dough resting time, cutting strength increased up to 30 minutes thereafter it started to decrease. Similar results were reported for the noodles with the dough resting time by Pronyk *et al.* (2007).

Table 4.31: Effect of dough resting time and different levels of baking powder on the spread ratio and hardness of 25% GRP biscuit

| | Baking powder (%) *Resting time (min) | 20 | 30 | 40 |
|-------------------------|--|-------------------------|-------------------------|-------------------------|
| Spread ratio | 0.25 | 4.66±0.09 ^{aA} | 4.53±0.19 ^{aA} | 4.50±0.24 ^{aA} |
| | 0.5 | 4.89±0.16 ^{aA} | 4.84±0.17 ^{aA} | 4.74±0.28 ^{aA} |
| | 0.75 | 5.09±0.40 ^{aA} | 5.32±0.31 ^{aB} | 5.46±0.41 ^{aB} |
| Cutting strength (N) | 0.25 | 7.00±0.39 ^{aA} | 7.22±0.51 ^{aA} | 7.56±0.35 ^{aA} |
| | 0.5 | 7.93±0.55 ^{aB} | 8.59±0.77 ^{aB} | 7.22±0.70 ^{aA} |
| | 0.75 | 6.87±0.64 ^{aA} | 7.45±0.36 ^{aA} | 6.91±0.17 ^{aA} |

Data are represented as mean±S.D (n=5)

^a indicates within a baking powder level mean with superscripts are significantly different (P<0.05)

^{AB} indicates within a dough resting time with different superscripts are significantly different (P<0.05) from each other

Table 4.32: p-values of main effects and interaction effects for spread ratio and hardness

| Parameter | Spread ratio | cutting strength |
|-----------------------------|----------------------|------------------|
| Baking powder | <0.001* | <0.001* |
| Resting time | <0.975 ^{NS} | <0.02* |
| Baking powder* Resting time | <0.17 ^{NS} | <0.018* |

p-value with superscript * and ^{NS} indicates that independent variables or its interaction variables or its interaction have a significant (p<0.05) & non-significant(p>0.05) effect on dependent variables respectively

4.5 Analysis of batter and muffin

4.5.1 Colour and viscosity of the batters

The lightness of wheat batter (W) was 68.90, WB was 63.32 and by replacing whey protein concentrate (WPC) with the ghee-residue powder, the lightness of the batter decreases from 63.32 to 50.73. When refined wheat flour was replaced by 50 % with the pearl millet flour, the redness value increased to 1.06 and when we replace the WPC with ghee-residue powder, the redness value increased from 4.02 to 8.32 significantly ($p < 0.05$). The yellowness value of wheat batter was 24.34, when it was substituted with pearl millet flour yellowness decreased to 23.62; when WPC was replaced with ghee-residue powder the yellowness began to increase from 23.32 to 26.20.

The amount of air incorporated in the batter is indirectly indicated by the specific volume of the batter (Khalil, 1998). This attribute eventually leads to the desirable spongy, fluffy, and high-volume product. The specific volume of the refined wheat flour batter (W) was lower; however, when pearl millet was substituted for refined wheat flour, the specific volume increased to 1.05 cm³/g, and further, the specific volume was increased when WPC was replaced with ghee residual powder. According to Turabi *et al.* (2008), the smaller the specific volume of batter, the higher amount of air will be incorporated during baking.

Table 4.33: Effect on colour and viscosity of muffin batter upon GR substitution

| Batter sample | L* | a* | b* | Specific volume (cm ³ /g) |
|---------------|-------------------------|------------------------|--------------------------|--------------------------------------|
| W | 68.90±0.26 ^f | 0.08±0.01 ^a | 24.34 ±0.10 ^b | 0.99±0.02 ^a |
| WB | 63.32±0.28 ^e | 1.06±0.09 ^b | 23.62±0.08 ^a | 1.05±0.01 ^c |
| WB25 | 60.37±0.14 ^d | 4.02±0.12 ^c | 23.32±0.10 ^a | 0.99±0.01 ^a |
| WB50 | 56.58±1.95 ^c | 4.94±0.06 ^d | 24.56±0.11 ^b | 1.04±0.01 ^{bc} |
| WB75 | 53.24±0.90 ^b | 7.86±0.10 ^e | 27.79±0.04 ^d | 1.02±0.00 ^{ab} |
| WB100 | 50.73±0.28 ^a | 8.32±0.05 ^f | 26.20 ±0.20 ^c | 1.03±0.02 ^{bc} |

Data are represented as mean±S.D (n=3)

^{abcdef} Means with different superscript in different rows of a column differ significantly ($p < 0.05$)

Note: W-Batter from RWF, WB- Batter from RWF and PMF (50:50), WB25- Batter from RWF and PMF (50:50) with 25% replacement of WPC, WB50- Batter from RWF and PMF (50:50) with 50% replacement of WPC, WB75- Batter from RWF and PMF (50:50) with 75% replacement of WPC, WB100-Batter from RWF and PMF (50:50) with 100% replacement of WPC.

4.5.2 Determination of flow properties of muffin batter

4.5.2.1 Viscosity as a function of shear rate

In Table 4.34, the wheat batter (W) had the highest viscosity among all the batters, as well as the lowest specific volume. As a result, foam stability and air incorporation will be improved. However, the viscosity was lowered when refined wheat flour was replaced with pearl-millet flour. The obtained result could be related to the system's dilution of refined wheat flour protein, which is a key component for mixing structure development (Loewe, 1993).

Table 4.34: viscosity of the muffin batters

| | W | WB | WB25 | WB50 | WB75 | WB100 |
|-----------|-------------------------|------------------------|------------------------|------------------------|------------------------|--------------------------|
| Viscosity | 13.83±1.87 ^b | 9.04±1.83 ^a | 9.22±1.51 ^a | 7.08±1.87 ^a | 8.24±0.54 ^a | 10.71±1.43 ^{ab} |

Data are represented as mean±S.D (n=3)

^{ab} Means with different superscript in different columns of row differ significantly (p<0.05)

The apparent viscosity was measured as a function of shear rate in the range of 0.1–1000 1/s with 100 points at 25°C. Figure 4.9 demonstrates the apparent viscosity vs shear rate for muffin batters made with different levels of WPC substitution with GRP. With the application of shear rate, all of the batters showed shear thinning behaviour (Baixauli, 2008).

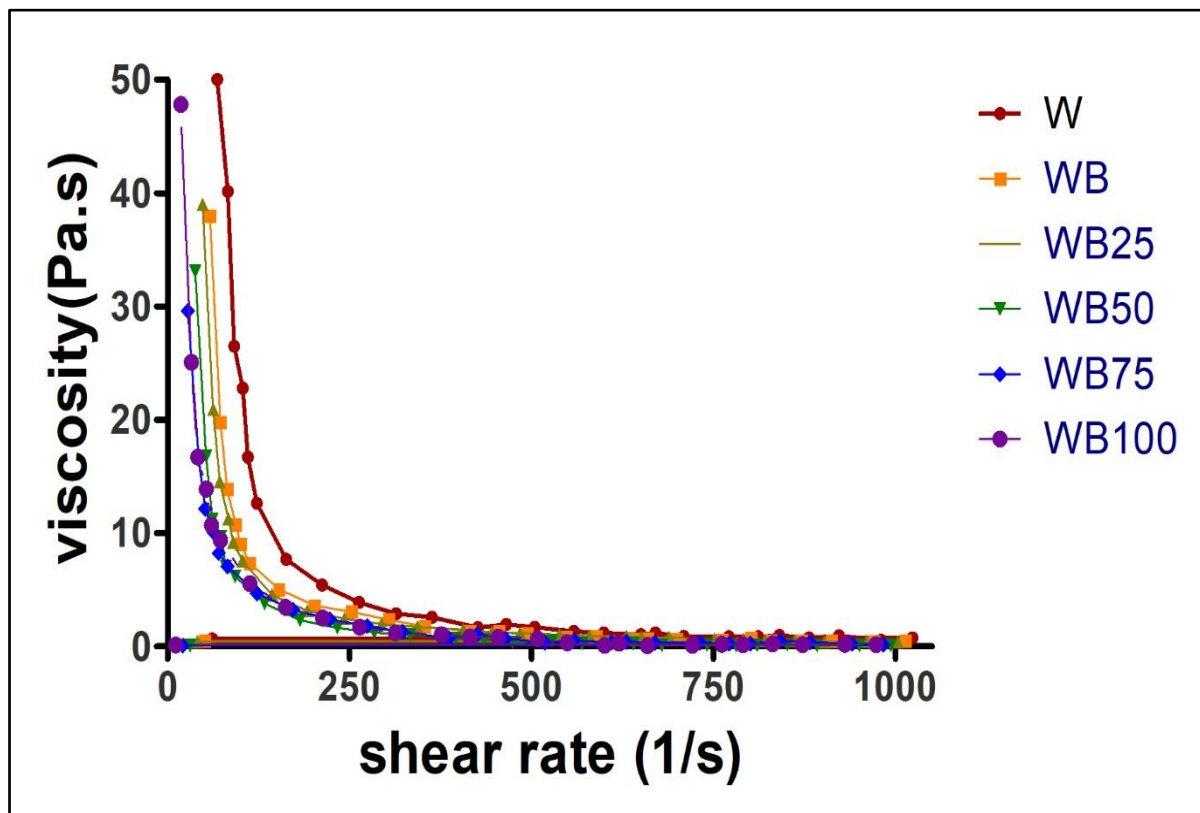


Fig 4.9: Influence of substituting WPC with different levels of GRP on flow properties of a muffin batter at 25°C

4.5.2.2 Viscosity as a function of temperature

The viscosity of the muffin batter was examined over a temperature sweep from 25 to 90 °C to determine the gelatinization temperature in the batter during baking. The viscosity of all the batters reduced as the temperature was increasing. The viscosity began to rise after 80°C, indicating the commencement of starch gelatinization (Shelke *et al.*, 2008). A decrease in the end product volume has been linked to lower batter viscosity during heating. During the heating process, higher viscosities reduce coalescence, migration, and loss of air cells before the batter sets. Higher viscosities during heating would allow the batters to retain better form by expanding air nuclei and resisting starch granule settling, resulting in improved cake volume and crumb grain (Baixauli, 2008).

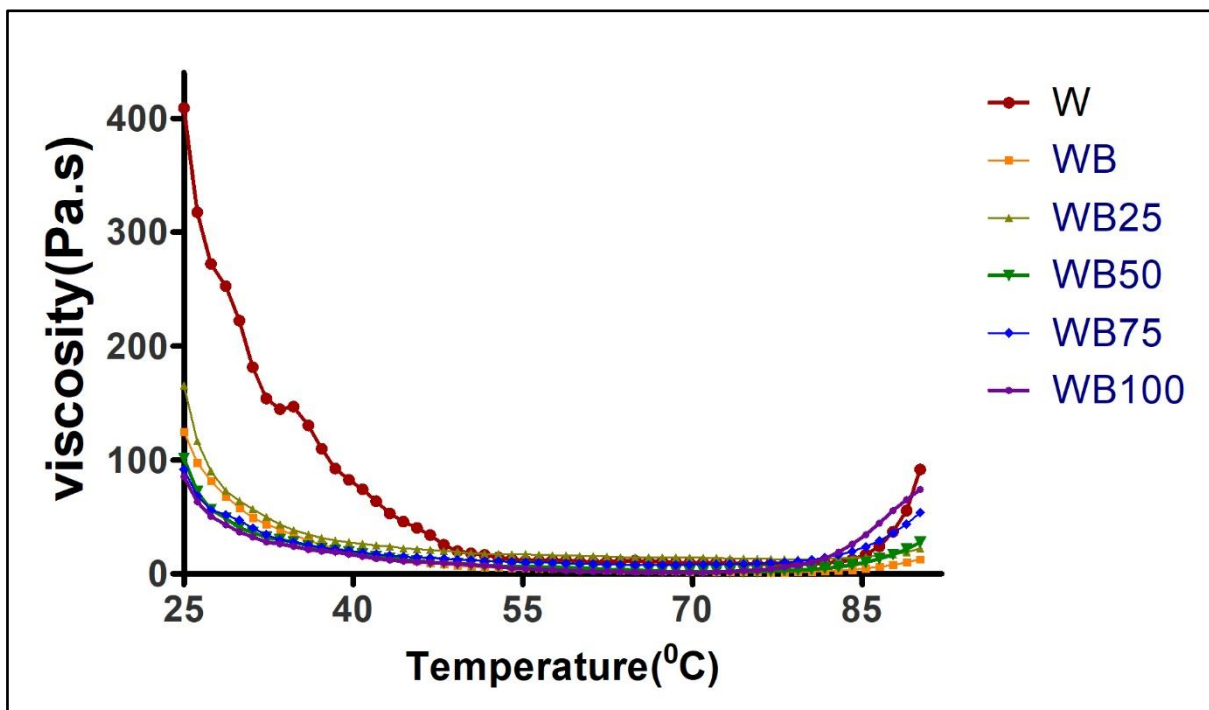


Fig 4.10: Evolution of viscosity during heating

4.5.3 Viscoelastic properties of different batter during heating

The linear viscoelastic characteristics of the muffin batter were examined over a temperature sweep from 25 to 85°C to approximate structural changes in the batter during baking. G' indicates the storage modulus, G'' indicates the loss modulus and $\tan \delta$ indicates an overall viscoelastic response of the material. The G' , G'' and tangent δ were plotted against the temperature by keeping the constant frequency (1Hz) as shown in figure 4.11 & 4.12. Both the storage modulus and the loss modulus were dropped when the temperature rose from 25 to 85°C. However, when the temperature was raised to 30°C, both moduli increased. Ngo &

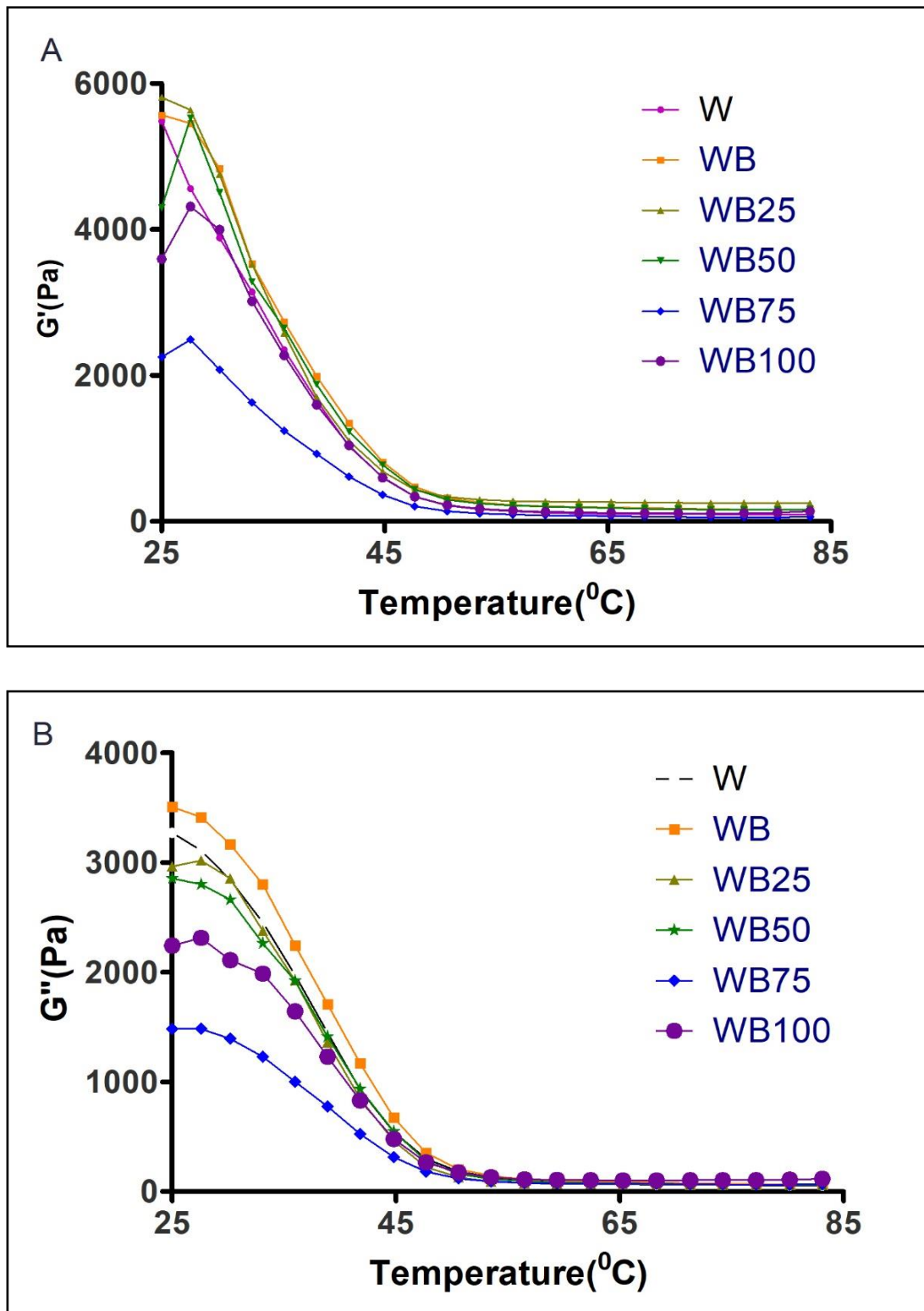


Fig 4.11: Influence of GRP levels in the muffin batter on the evolution of G' and G'' with temperature. Heating rate: $0.032^{\circ}\text{C}/\text{s}$. Strain wave amplitude: 0.05 (W, WB, WB25, WB50, WB75 and WB100). Frequency: 1 Hz (A) Storage modulus and (B) Loss modulus

Taranto (1986) noticed a similar tendency, where they associated this effect could be possibly due to protein-protein interactions. These results obtained were in agreement with the observations of Baixauli (2008). The storage modulus of all the batters was greater than the loss modulus, indicating soft gel behaviour. When compared to all other batters, WB75 & WB100 showed lower storage modulus, indicating that the batter was too liquid in nature.

It was stated that lower $\tan \delta$ values for refined wheat flour-based batter indicate a batter that was more elastic, stronger and time-stable (Herranz, 2016). The consistency of the batter determines the ultimate quality of the muffins. Lower batter viscosities during heating have been associated with lower volume in final products (Shelke *et al.*, 1990). Figure 4.12 shows that, except for the wheat flour batter (W), all other batters had a greater tangent delta, indicating that they behaved viscously at 85 °C, which is undesirable. Further, Herranz (2016) also mentioned that the higher $\tan \delta$ values ($\tan \delta$ near to 1) indicated that the protein was not heat stable. In this regard, substituting pearl-millet flour for refined wheat flour dilutes the gluten protein, and substituting whey protein concentrate for GRP causes the batter to behave highly viscous even at higher temperatures.

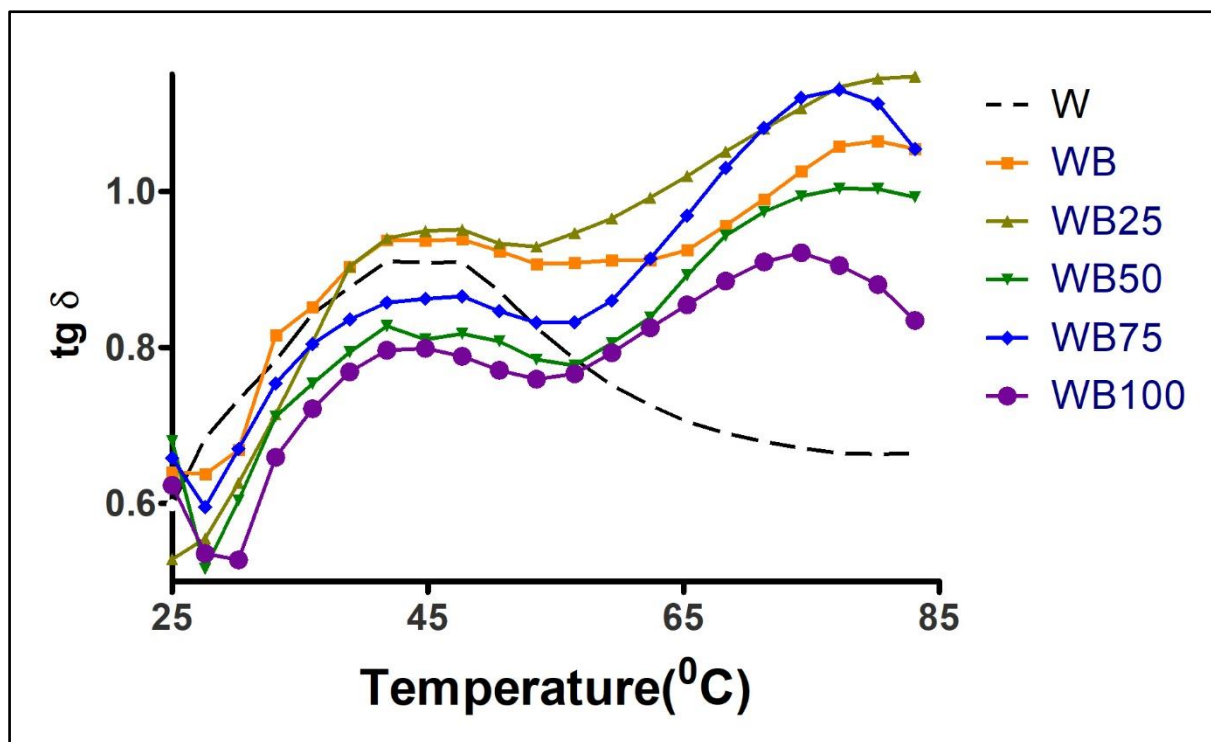


Fig 4.12: Influence of GRP levels in the muffin batter on the evolution of loss of tangent values ($\tan \delta$) with temperature. Heating rate: 0.032°C/s. Strain wave amplitude: 0.05 (W, WB, WB25, WB50, WB75 and WB100). Frequency: 1 Hz

4.5.4 Analysis of muffin

4.5.4.1 Proximate analysis of muffin

The proximate composition of the muffin is given in Table 4.35. the highest moisture was retained in the WB75 muffin and the lowest in the Pearl millet (WB) muffin. The fat levels were increased when WPC was replaced with ghee-residue powder due to the presence of high fat in ghee-residue powder. Protein content was found to be decreased with the replacement and this could be due to low protein content in GRP when compared with WPC. Ash was also found to be increasing with an increase in the degree of WPC substitution with GRP. These results obtained were in accordance with Sharoba (2021) who reported on pearl millet flour-based cake.

Table 4.35: Effect of different levels of GRP on the proximate values of eggless pearl millet-based muffin

| Treatments | Moisture (%) | Fat (%) | Protein (%) | Ash (%) | Acid insoluble ash |
|------------|--------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| W | 24.14±1.82 ^{ab} | 18.69±0.52 ^c | 9.68±0.03 ^f | 0.89±0.03 ^a | 0.07±0.00 ^{ab} |
| WB | 20.77±0.52 ^a | 16.68±1.15 ^a | 8.94±0.05 ^d | 0.96±0.01 ^{ab} | 0.06±0.01 ^a |
| WB25 | 23.78±0.49 ^{ab} | 17.45±0.35 ^b | 9.22±0.03 ^e | 1.00±0.01 ^{ab} | 0.07±0.00 ^b |
| WB50 | 24.01±1.19 ^{ab} | 18.76±0.51 ^c | 8.09±0.03 ^c | 1.07±0.06 ^{bc} | 0.08±0.01 ^c |
| WB75 | 25.31±1.49 ^b | 20.12±0.48 ^d | 7.64±0.10 ^b | 1.18±0.05 ^c | 0.09±0.01 ^d |
| WB100 | 24.10±1.60 ^{ab} | 20.37±0.08 ^d | 6.68±0.05 ^a | 1.20±0.08 ^c | 0.10±.00 ^e |

Data are represented as mean±S.D (n=3)

^{abcdef} Means with different superscript in different rows of a column differ significantly (p<0.05)

4.5.4.2 Crumb color and water activity of eggless Pearl millet-based muffin

The instrumental values for lightness (L*) and color (a*, b* value) for muffin samples are shown in Table 4.36. When refined wheat flour was substituted with pearl millet flour, the lightness values reduced from 67.55 (W) to 53.21 (WB) which could be to the contribution of dullness from PMF.

Umashankar *et al.* (2015) also reported similar findings. The lightness of the crumb was further reduced when WPC was replaced with GRP. The redness of muffins ranged from 0.71 to 10.66, and it increased when the amount of ghee-residue powder was increased. With GRP substitution, yellowness increased up to a level of 50% replacement, and then significantly decreased (p<0.05). WB75 had the highest water activity, while WB25 had the lowest. The determination of a_w is crucial to ensure the shelf life of muffins and consumer acceptability.

Table 4.36: Effect on colour and water activity of muffin upon GRP substitution

| Treatments | L* | a* | b* | a _w |
|------------|-------------------------|-------------------------|--------------------------|---------------------------|
| W | 67.55±0.13 ^f | 0.71±0.09 ^a | 20.72±0.16 ^{bc} | 0.903±0.002 ^{ab} |
| WB | 53.21±0.12 ^e | 1.41±0.04 ^b | 20.07±0.22 ^b | 0.904±0.013 ^{ab} |
| WB25 | 41.58±0.29 ^d | 5.13±0.09 ^c | 25.54±0.26 ^c | 0.891±0.011 ^a |
| WB50 | 39.38±0.56 ^c | 6.27±0.06 ^d | 21.07±0.27 ^a | 0.908±0.014 ^{ab} |
| WB75 | 34.95±0.48 ^b | 7.11±0.12 ^e | 20.01±0.41 ^b | 0.914±0.015 ^b |
| WB100 | 32.36±0.28 ^a | 10.66±0.06 ^f | 16.28±0.35 ^a | 0.902±0.012 ^{ab} |

Data are represented as mean±S.D (n=3)

^{abcdef} Means with different superscript in different columns of a row differ significantly (p<0.05)

4.5.4.3 Physical properties of muffin

The weight loss of a muffin prepared using refined wheat flour (W) was ~12.14 %, and then the weight loss was reduced to ~11.91 % when refined wheat flour was substituted with pearl millet flour, although the difference was statistically insignificant (p<0.05). According to Bhaduri (2013), moisture loss was greater in flours with lower gluten concentration due to a lack of water binding capacity and Thalor (2021) stated that the water binding capacity of GRP is around 2.2 g/ g of protein. The moisture retention in the WPC-replaced muffins may be impacted by the presence of GRP. When refined wheat flour was replaced with pearl-millet flour, the volume of the WB muffin was lowered to ~51.47 cm³. This could be due to lower levels of gluten protein. When WPC is replaced with GRP, the volume of the muffins decreases, either due to a higher specific volume value or fewer air cells incorporated and inadequate air bubble retention capacity of the muffin batter (Khalil, 1998). According to Gomez *et al.* (2008), the volume of a muffin can also be determined by the amino acid composition of the flour and the amount of protein.

Table 4.37: Effect of substitution of ghee-residue powder on Physical properties of muffin

| Treatments | Weight loss (%) | Volume (cm ³) |
|------------|---------------------------|---------------------------|
| W | 12.14±0.65 ^a | 56.19 ±8.56 ^c |
| WB | 11.91±0.09 ^a | 51.47±3.37 ^{bc} |
| WB25 | 13.35±0.38 ^{bc} | 42.19±5.35 ^a |
| WB50 | 12.63±0.79 ^{ab} | 44.48±3.79 ^{ab} |
| WB75 | 12.74±0.58 ^{abc} | 45.17±4.01 ^{ab} |
| WB100 | 13.68±0.50 ^c | 36.84±0.50 ^a |

Data are represented as mean±S.D (n=6)

^{abc} Means with different superscript in different rows of a column differ significantly (p<0.05)

4.5.4.4 Sensory evaluation of muffin

The refined wheat flour muffin had very fine cells and a smooth, fluffy texture. Refined wheat flour muffins (W) received the highest score in color and appearance, taste and texture, and overall acceptability. The flavor and sweetness of the WB muffin were slightly enhanced when refined wheat flour was replaced with pearl millet flour, but not drastically. All sensory attributes were decreasing when WPC was replaced with ghee-residue powder. The body of the muffin collapsed when more than half of the WPC was replaced with ghee-residue powder, while WB75 and WB100 had surface cracking in the crust, rendering them unsuitable for muffin production. The ghee-residue powder was only able to replace up to 50% of the WPC. Ghee residue or ghee residue powder can be effectively utilized in formulation of biscuits or muffins.

Table 4.38: Effect of substitution of GR on sensory parameters and overall acceptability of muffin

| Sensory attributes | W | WB | WB25 | WB50 | WB75 | WB100 |
|-----------------------|----------|-----------|-----------|-----------|-----------|-----------|
| Colour & Appearance | 8.25±0.5 | 7.60±0.55 | 7.50±1.12 | 7.10±1.24 | 7.40±0.55 | 7.40±0.89 |
| Body & Texture | 8.00±0.0 | 7.60±0.55 | 7.60±0.84 | 7.60±1.14 | 7.40±1.14 | 7.30±1.30 |
| Flavour | 7.87±0.4 | 8.00±0.00 | 7.90±0.89 | 7.80±1.30 | 7.60±1.08 | 7.10±0.74 |
| Sweetness | 7.87±0.8 | 8.00±0.00 | 7.90±0.89 | 7.40±1.14 | 7.60±1.14 | 7.30±0.84 |
| Overall acceptability | 8.00±0.4 | 7.80±0.45 | 7.95±0.87 | 7.30±0.97 | 7.25±0.90 | 7.10±0.74 |

* Data are represented as mean±S.D (n=5)

^a Means with a different superscript in different columns of a row differ significantly (p<0.05)

4.5.4.5 Textural properties of muffin

The Table 4.39, it shows the effect of GRP on the textural qualities of eggless pearl millet-based muffins. All textural qualities reduced significantly (p<0.05) when refined wheat flour was replaced with pearl millet flour. All textural qualities fell drastically when WPC was replaced with GRP, except the hardness of WB25. The muffins were less elastic due to a decrease in cohesiveness, springiness, and resilience. Lower resilience denotes that the muffins were less spongy, as they take a longer duration to recover after compression (Goswami, 2015). Springiness is related to fresh, aerated product, hence high springiness values indicate good muffin quality (Sanz *et al.*, 2009).

Table 4.39: Effect of substitution of ghee-residue powder on textural properties of muffin

| Textural characteristics | W | WB | WB25 | WB50 | WB75 | WB100 |
|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------|
| Adhesiveness (g.s) | -0.85±0.41 ^a | -0.66±0.29 ^a | -1.17±0.27 ^a | -1.86±1.96 ^a | -2.00±1.29 ^a | -1.28±0.55 |
| Springiness (mm) | 0.56±0.07 ^{cd} | 0.59±0.05 ^d | 0.50±0.03 ^{bc} | 0.46±0.03 ^b | 0.32±0.01 ^a | 0.25±0.01 |
| Cohesiveness | 0.48±0.04 ^d | 0.39±0.05 ^c | 0.34±0.01 ^{bc} | 0.31±0.03 ^b | 0.19±0.02 ^a | 0.17±0.00 |
| Gumminess (N) | 1.59±0.1 ^d | 1.12±0.42 ^{bc} | 1.51±0.10 ^{cd} | 0.83±0.16 ^{ab} | 0.52±0.08 ^a | 0.40±0.19 |
| Chewiness (N.mm) | 0.90±0.17 ^b | 0.68±0.28 ^b | 0.75±0.07 ^b | 0.38±0.01 ^a | 0.06±0.01 ^a | 0.10±0.05 |
| Resilience | 0.17±0.02 ^c | 0.14±0.02 ^c | 0.11±0.01 ^b | 0.10±0.01 ^b | 0.06±0.01 ^a | 0.05±0.00 |
| Hardness (N) | 3.31±0.21 ^c | 3.15±0.29 ^c | 4.43±0.29 ^d | 2.62±0.28 ^b | 2.63±0.21 ^b | 1.82±0.23 |

Data are represented as mean±S.D (n=5)

^{abcd} Means with different superscript in different columns of a row differ significantly ($p < 0.05$)



W

WB

WB25

WB50

WB75

WB100

Fig 4.13: Muffin made from different batter composition



CHAPTER 5



SUMMARY AND CONCLUSION

5.0 Summary and Conclusion

Nowadays bakery products are becoming an essential part of most people's meals. Modernization, changes in lifestyle, working women, availability of convenient and fast foods in the market and insufficient time to cook the food led to an increase in the consumption of bakery products. Realizing the role of low sugar products, low fat, high protein and fibre-rich products on human health, most of the innovations in bakery sector are focussing on novel formulations.

Wheat is usually utilized to make bakery products which may pose allergenicity in certain individuals and lead to celiac diseases. Hence the consumers are looking for alternatives to wheat. Pearl millet (PM) is major millet crop world-wide and India is its largest producer. It used to be the major staple before the green revolution but later on area, production and consumption has declined substantially. There is revitalized interest among the stakeholders regarding the nutritional and therapeutic virtues of Pearl millet and other millet crops. Compositional superiority and richness in phytochemicals in comparison to staple cereals provided an opportunity to develop newer range of food products using millets. Pearl millet has antioxidant, anti-inflammatory and anti-diabetic properties. Pearl millet is recommended for diabetic patients because of its low glycemic index and richness in fibre. Pearl millet is used to prepare many traditional food products like porridge, fura and flatbreads, *khidadi*, kheer and beverages which are usually consumed by ethnic people. However, there is need to develop technological interventions to overcome the difficulties in processing, removal of anti-nutritional factors and improving the storage stability of PM based primary and secondary processed products.

Ghee-residue (GR) is a dairy by-product obtained during the manufacturing of ghee, which is a moist brownish in colour and rich in denatured proteins and phospholipids with entrapped fat. Majority of ghee residue is discarded or utilized for preparation of value added products at small scale. Attempts have been made to develop ghee residue powder by admixing it with water or skim milk or buttermilk and developed ghee residue powder (GRP) exhibits excellent functional properties besides offering vital nutrients. GR or GRP can be utilized for the partial replacement of bakery fat or milk proteins from the formulation of biscuits and muffins. Replacement of these major ingredients may alter the product quality characteristics. Bakery fat or shortening performs number of functions in bakery products and essential for producing excellent quality products. The bakery fat replacement with other fat-

rich ingredients such as GR or GRP will influence the rheological properties of the dough/batter and also the overall quality attributes of the bakery products. Preliminary investigations carried out in our laboratory indicated that GR or GRP incorporation at higher levels often leads to textural defects and optimization of their level and process modification is required to develop acceptable quality products.

Hence the present investigation was carried out with the objective to investigate the effect of GR or GRP on the replacement of bakery fat and whey protein concentrate (WPC), from biscuit and muffin formulation, respectively on their rheological, textural, physico-chemical and sensory qualities. The effect of baking powder and dough resting times is also determined. The key findings of the experiments were briefly summarized below.

5.1 Composition of Raw Materials

Proximate analysis of Pearl millet flour and refined wheat flour indicated that the pearl millet flour had higher levels of fat, protein and ash in comparison to the refined wheat flour. Pearl millet and refined wheat flour had a moisture, fat, protein & ash of 6.53, 5.62, 11.14 & 1.66 and 9.33, 1.67, 5.88 & 0.65 % respectively. The chemical composition of ghee residue and ghee residue powder showed that it has % total solid, % fat, % protein, % ash & % lactose of 28.19, 8.18, 12.18, 1.49 & 6.32, and 99.08, 46.09, 31.18, 4.91 & 16.55, respectively.

5.2 Ghee Residue substitution on properties of biscuit dough

The lightness and yellowness of the dough were reduced with the substitution of GR due to the presence of Maillard reaction products in GR. The water activity of the dough decreased with the addition of ghee residue. Bakery fat substitution with GR resulted in decrease in hardness and gumminess linearly ($p < 0.05$) except for resilience, which increased non-significantly ($p > 0.05$). The other textural parameters also decreased but there was no consistent trend for adhesiveness, cohesiveness, springiness and chewiness and the dough made with GR @15 percent exhibited lowest values ($p < 0.05$) for above-mentioned parameters.

Shortening replacement with the ghee residue resulted in maximization of strain but lowering in recovery of dough samples. The frequency sweep test showed that the dough samples had a higher storage modulus than the loss modulus indicating that the dough was viscoelastic in nature. Dynamic viscoelastic loss factor showed WBGR25 dough sample had higher tangent δ indicated dough was less elastic compared to other doughs. The highest

elastic recovery was due to the high protein content in flour; however, when the GR was incorporated into the dough, it resulted in increased moisture content of the dough; rheological analysis revealed that WBD50 dough had the highest recovery and percentage recovery when compared to all other doughs

5.3 Ghee Residue Substitution: Effect on biscuit Quality Attributes

There was no significant difference in the biscuit with the addition of ghee residue however; the ash content increased significantly. The lightness of biscuits was enhanced by adding ghee residue, while the redness and yellowness lowered down. The browning index was reduced with the addition of ghee residue. The spread ratio and hardness/cutting strength of biscuits increased with the addition of ghee residue. Descriptive sensory analysis showed that the control sample had the highest sensory score than the GR substituted ones. Among the ghee residue substituted biscuits, biscuits with 20% GR scored maximum for overall acceptability. The 20% GR incorporated biscuit had moisture, fat, protein and ash of 1.01, 19.21, 6.44 & 1.19 % respectively. The spread ratio and hardness of the 20% GR biscuit were 6.12 & 32.46 N respectively.

Further, to analyze the interaction effect of dough resting time and baking powder level on the organoleptic quality biscuit i.e., 20% GR was selected for further investigation. The lightness value increased as the dough resting time and baking powder level was raised. The redness and yellowness, on the other hand, decreased. As the resting time of the dough increased, the water activity decreased probably due to the higher water binding by the flour constituents and other ingredients. The biscuit spread ratio also enhanced with the dough resting time up to 30 minutes then it dropped. The dough resting time had no effect on the hardness of a biscuit, while an increase in the baking powder level led to lowering in hardness which can be attributed to leavening effect and production of CO₂ that make the biscuits porous. Descriptive analysis of the biscuits samples by the panellists indicated that 0.75 % baking powder and 20 minutes of dough resting time was optimum parameters for the GR incorporated PM-RWF composite-based biscuits.

5.4 Ghee-residue powder (GRP) addition: Effect on Dough and Biscuits Properties

Lightness, yellowness and redness values decreased on bakery fat substitution with ghee residue powder. The water activity of the dough decreased with the increased level of substitution with ghee residue powder. The hardness of the dough increased significantly due to the addition of ghee residue powder. All the dough samples exhibited viscoelastic

behaviour when constant stress was applied. All the dough sample's maximum strain was similar to the control, however, GRP10 had a lower maximum strain. Compared to control, the percentage recovery was high for all the ghee residue powder added to doughs. The storage modulus and loss modulus were decreasing with the addition of ghee residue powder compared to WBD50. The tangent delta of the ghee residue powder added samples were higher than the WBD50 dough sample.

5.5 The effect of ghee-residue powder on the quality parameters of biscuits

The ghee residue powder added to biscuits had moisture ranging from 1.44 % to 2.49 %, fat 17.34 % to 23.44 %, protein ranged from 6.72 % to 11.30 % and ash content was in the range of 1.03 % to 1.23 %. The lightness and yellowness of the biscuits lowered with the addition of ghee residue powder while the redness was increased. The water activity of all the ghee residue powder added samples were increasing. The spread ratio was decreased with an increase in the level of substitution with ghee residue powder. The cutting strength of the biscuits increased with an increase in the level of substitution with ghee residue powder. Descriptive analysis showed that 25% GRP biscuit has the highest overall acceptable score compared to others. The chemical composition of the 25% GRP biscuit i.e., it has moisture, fat, protein, and ash content of 1.44, 23.44, 11.33 & 1.23 % respectively. The spread ratio was minimum in the 25% GRP biscuit and the cutting strength was 84.24 N.

The sensorily accepted biscuit was further analysed to optimize the baking powder and dough resting time. Lightness and yellowness values increased up to 30 minutes of dough resting time thereafter decreased and lightness & yellowness decreased up to 0.5 % baking powder level thereafter increased. The spread ratio was increased with an increase in baking powder level and also with dough resting time. Hardness was increased with dough resting time. Descriptive analysis showed that the 0.5 % baking powder and 30 minutes of dough resting time had the highest sensory score.

5.6 Quality of batter and muffin

The batter lightness was decreasing with the increase in substitution of WPC with the ghee residue powder, while redness and yellowness were increasing. The specific volume of the muffin batter was found to be increasing with the replacement of WPC with GRP. The viscosity of the batter increased with the substitution of refined wheat flour with pearl millet flour and a similar trend was found with the replacement of WPC with the ghee residue powder. The flow behaviour showed with the application of shear rate all the batter showed

shear thinning behaviour. The viscosity of the batter was also evaluated during temperatures between 25 to 90°C to approximate the gelatinization. The viscosity of all the batters decreased up to 80°C thereafter it began to rise indicating the commencement of gelatinization. The frequency sweep test showed all the batters behave as a soft gel. The wheat batter (W) showed a lower tangent delta indicating it is more elastic, stronger and time stable. When WPC was substituted with the ghee residue powder, the tangent delta was increased due to unstable proteins.

It was observed that the fat content was increased when replacing WPC with ghee-residue powder due to the high fat of ghee-residue powder. Protein content was decreasing with the replacement of WPC with ghee residue powder due to low protein content in ghee residue powder compared to WPC. Ash content was increasing with an increase in the level of ghee residue powder substitution. The L^* value decreased with the addition of ghee residue powder while a^* & b^* were increased. The lowest weight loss was recorded for wheat & pearl millet batter (WB) and the highest volume was recorded in muffins made out of refined wheat flour. Instrumental textural properties from the double bite test showed that the WB25 muffin had maximum hardness. With the replacement of WPC with the ghee residue powder, springiness, cohesiveness, gumminess chewiness and resilience were decreased significantly. Hedonic scale sensory evaluation showed that the wheat muffin (W) had the highest sensory score and among the muffins with ghee residue powder, WB25 had the highest sensory score.

Based on the above-mentioned findings it can be concluded that both ghee residue and ghee residue powder can be utilized to substitute bakery fat and milk proteins in the formulation of biscuits and muffins, respectively. The incorporation or substitute of GR and GRP influenced the characteristics of dough or batter as well as quality of resultant products. The addition of GR or GRP not only provide milk fat, proteins and phospholipids but improve the colour properties of bakery products. On many aspects the functionality offered by ghee residue or ghee residue powder is similar to cocoa powder. However, there is further scope for improving their functionality by modifying their production process. GR or GRP incorporation may also be feasible with other bakery products and research efforts should be focussed on substituting it wherever cocoa powder is utilized as ingredient.



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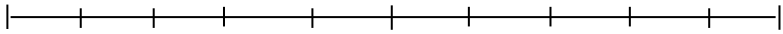
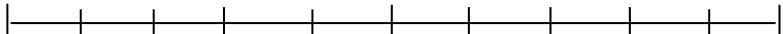


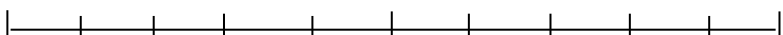
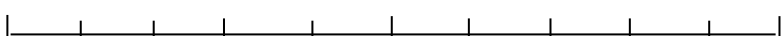


APPENDICES


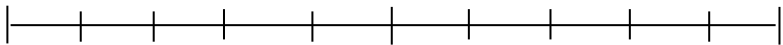
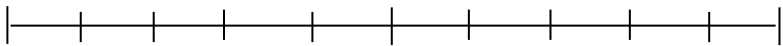


APPENDEX-I
Sensory Evaluation Card for Pearl millet- based Biscuit

Sample: _____

Date: ___/___/2021

Kindly evaluate the given samples sensorily using the intensity scales. To indicate your judgment, write the sample code in respective cell.

| Property | Intensity |
|-------------------|--|
| Appearance | |
| Powdery surface |  Smooth 5 Extreme |
| Browning |  Nil 5 Extreme |
| Flavour | |
| Raw |  Nil 5 Extreme |
| Toasted |  Nil 5 Very intense |
| Rancid |  Nil 5 Extreme |
| Foreign flavour |  Nil 5 Extreme |
| Sweet |  Nil 5 Extreme |
| Texture | |
| Crispness |  Nil 5 Extreme |

| | |
|-----------------------|--|
| Hardness |  Nil 5 Extreme |
| Coarseness |  Nil 5 Extreme |
| Chewiness |  Nil 5 Extreme |
| Adhesiveness |  Nil 5 Extreme |
| Overall Acceptability |  Disliked 5 Liked |

Remarks, if any:

Signature

Name

| Attribute name | Attribute definition |
|------------------------------|--|
| Appearance | |
| Powdery surface | The amount of powder released when both sides of the biscuit are gently rubbed with fingers. |
| Browning | The colours range from light cream/golden to dark brown. |
| Flavour | |
| Raw | A raw or starchy or underbaked odor attribute in the product uncooked or raw |
| Toasted | Aroma intensity associated with toasted/burnt |
| Sweetness | Intensity of the product's sweet taste |
| Rancid | Aroma intensity associated with rancid/old oil |
| Foreign flavour | Experiencing a flavor that is not characteristic of the product |
| Texture | |
| Hardness | Biscuit hardness/softness as assessed between the incisors |
| Crispiness | When bitten, the force and sound with which the biscuits break |
| Coarseness | The degree to which the inside of the mouth feels grainy/coarse while masticating |
| Chewiness | Energy and time needed to masticate a product to a ready-to-swallow state |
| Adhesiveness | The amount of product that adhered to the teeth/palate during mastication. |
| Overall acceptability | The product's overall sensorial likeness or dislike based on all sensorial attributes evaluated. |

APPENDEX-II

SCORE CARD FOR SENSORY EVALUATION OF MUFFINS

DT Division, NDRI, KARNAL

Date:

Please evaluate the given samples of *Muffins* using 9-point Hedonic scale.

| Hedonic Rating Score | Score |
|-----------------------------|--------------|
| Like Extremely | 9 |
| Like Very Much | 8 |
| Like Moderately | 7 |
| Like Slightly | 6 |
| Neither like nor dislike | 5 |
| Dislike Slightly | 4 |
| Dislike Moderately | 3 |
| Dislike Very Much | 2 |
| Dislike Extremely | 1 |

Score of samples

| Sensory attributes | Product code | | |
|------------------------------|---------------------|--|--|
| | | | |
| Colour and appearance | | | |
| Body & Texture | | | |
| Flavour | | | |
| Sweetness | | | |
| Overall acceptability | | | |

Suggestion/ Remarks:

Signature: _____

Name: _____