THE PRODUCTION OF A FUNCTIONAL YOGURT FORTIFIED WITH BROWN RICE

ESMER PİRİNÇLE ZENGİNLEŞTİRİLMİŞ FONKSİYONEL YOĞURT ÜRETİMİ

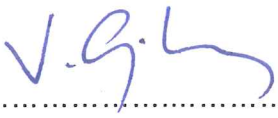
MUHAMMAD USMAN AKRAM

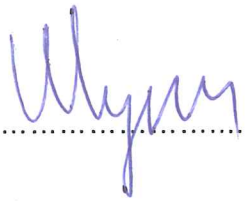
Prof. Dr. ÜMRAN UYGUN
Supervisor

Submitted to Graduate School of Science and Engineering of Hacettepe University
as a Partial Fulfillment to the Requirements
for the Award of the Degree of Master of Science
in Food Engineering

2018

This work named "**The Production of a Functional Yogurt Fortified with Brown Rice**" by **MUHAMMAD USMAN AKRAM** has been approved as a thesis for the Degree of **MASTER OF SCIENCE IN FOOD ENGINEERING** by the below mentioned Examining Committee Members.

Prof. Dr. Vural GÖKMEN
Head ..... 

Prof. Dr. Ümran UYGUN
Supervisor ..... 

Prof. Dr. Dilek SİVRİ ÖZAY
Member ..... 

Assoc. Prof. Dr. Birce MERCANOĞLU TABAN
Member ..... 

Assoc. Prof. Dr. Ali TOPCU
Member ..... 

This thesis has been approved as a thesis for the Degree of **MASTER OF SCIENCE IN FOOD ENGINEERING** by Board of Directors of the Institute for Graduate School of Science and Engineering.

Prof. Dr. Menemşe GÜMÜŞDERELİOĞLU
Director of the Institute of
Graduate School of Science and Engineering

YAYINLAMA VE FİKRİ MÜLKİYET HAKLARI BEYANI

Enstitü tarafından onaylanan lisansüstü tezimin/raporumun tamamını veya herhangi bir kısmını, basılı (kağıt) ve elektronik formatta arşivleme ve aşağıda verilen koşullarla kullanıma açma iznini Hacettepe üniversitesine verdiğimi bildiririm. Bu izinle Üniversiteye verilen kullanım hakları dışındaki tüm fikri mülkiyet haklarım bende kalacak, tezimin tamamının ya da bir bölümünün gelecekteki çalışmalarda (makale, kitap, lisans ve patent vb.) kullanım hakları bana ait olacaktır.

Tezin kendi orijinal çalışmam olduğunu, başkalarının haklarını ihlal etmediğimi ve tezimin tek yetkili sahibi olduğumu beyan ve taahhüt ederim. Tezimde yer alan telif hakkı bulunan ve sahiplerinden yazılı izin alınarak kullanması zorunlu metinlerin yazılı izin alarak kullandığımı ve istenildiğinde suretlerini Üniversiteye teslim etmeyi taahhüt ederim.

☐ **Tezimin/Raporumun tamamı dünya çapında erişime açılabilir ve bir kısmı veya tamamının fotokopisi alınabilir.**
(Bu seçenekle teziniz arama motorlarında indekslenebilecek, daha sonra tezinizin erişim statüsünün değiştirilmesini talep etseniz ve kütüphane bu talebinizi yerine getirse bile, tezinin arama motorlarının önbelleklerinde kalmaya devam edebilecektir.)

☐ **Tezimin/Raporumun ..... tarihine kadar erişime açılmasını ve fotokopi alınmasını (İç Kapak, Özet, İçindekiler ve Kaynakça hariç) istemiyorum.**
(Bu sürenin sonunda uzatma için başvuruda bulunmadığım taktirde, tezimin/raporumun tamamı her yerden erişime açılabilir, kaynak gösterilmek şartıyla bir kısmı ve ya tamamının fotokopisi alınabilir)

☒ **Tezimin/Raporumun 20.02.2020 tarihine kadar erişime açılmasını istemiyorum, ancak kaynak gösterilmek şartıyla bir kısmı veya tamamının fotokopisinin alınmasını onaylıyorum.**

☐ **Serbest Seçenek/Yazarın Seçimi**

12 / 02 / 2018

(İmza)

Muhammad Usman AKRAM

ETHICS

In this thesis study, prepared in accordance with the spelling rules of Institute of Graduate Studies in Science of Hacettepe University,

I declare that

- all the information and documents have been obtained in the base of the academic rules
- all audio-visual and written information and results have been presented according to the rules of scientific ethics
- in case of using others works, related studies have been cited in accordance with the scientific standards
- all cited studies have been fully referenced
- I did not do any distortion in the data set
- and any part of this thesis has not been presented as another thesis study at this or any other university.

12/02/2018

MUHAMMAD USMAN AKRAM

ABSTRACT

THE PRODUCTION OF A FUNCTIONAL YOGURT FORTIFIED WITH BROWN RICE

Muhammad Usman AKRAM

Master of Science, Department of Food Engineering

Supervisor: Prof. Dr. Ümran UYGUN

January 2018, 62 pages

In this study, functional yogurt with high γ-aminobutyric acid (GABA) content was produced by using crude extracts of germinated brown rice (GBR). GABA fortification by GBR is considered as easy, applicable and economical process for the development of functional yogurt.

Germination was achieved for 96 hours and rice was dried and grounded after germination. Effect of germination period on GABA production, total γ-oryzanol content and total antioxidant capacity was investigated for GBR. It was observed that GABA amount significantly increased with germination period and reached to almost 14 folds after 84 hours of germination. However, γ-oryzanol did not change significantly with germination. The total antioxidant capacity of GBR decreased in first 12 hours of germination then increased up to 84 hours with minor differences ($p < 0.05$).

GABA fortified functional yogurt samples at different levels were prepared and manufacturing conditions were optimized by using crude extracts obtained from 84 hours GBR flour. And these crude extracts having GABA contents of 13.88 ± 0.71 and 26.86 ± 0.34 mg/100 g dry matter were prepared from 20 and 40 g of flour samples, respectively. The effect of storage period and the addition of GABA standard or crude GBR extracts in all samples were determined by the comparison to control yogurt sample. On the other hand, naturally occurring GABA was not detected in control yogurt (CTR-Y).

Fermentation process results in GABA reduction of about 10.36, 14.39 and 18.57% in yogurt samples with GABA standard (CTR-G), crude extracts of 20 g GBR (GBR20) and crude extract of 40 g GBR (GBR40), respectively ($p < 0.05$). Whereas, storage period (21 days) has no significant effect on the GABA content of fortified yogurt samples (GABA content of CTR-G, GBR20 and GBR40 yogurt samples is 9.24 ± 0.07, 10.84 ± 0.14 and 21.78 ± 0.13 mg/100 g fresh yogurt, respectively). As compared to CTR-Y yogurt, water holding capacity (WHC) and textural parameters (hardness and gumminess) significantly decreased in GABA containing yogurt samples after 21 days of storage. However, during initial 14 days of storage, WHC and texture were first increased in all yogurt samples and then the reduction was observed during rest of the storage. In contrast, parameters such as cohesiveness and springiness were improved non-significantly for GABA enriched yogurt samples. Total antioxidant capacities of yogurt samples were increased with GABA fortification in the form of crude extracts but yogurt enriched with standard GABA has been observed with lower antioxidant capacity as compared to control yogurt ($p < 0.05$), whereas storage period has a non-significant effect on the antioxidant capacity of all yogurt samples.

The results of sensory evaluation revealed less acceptability for GBR40 yogurt than control yogurt but GBR20 has been selected as a suitable replacement for conventional yogurt. Furthermore, only a single portion of GBR20 yogurt is enough to supply more than 10 mg of GABA, which is enough for the treatment of individuals with mild hypertension according to the related literature.

Keywords: γ-aminobutyric acid, crude extract of germinated brown rice, functional yogurt, γ-oryzanol and total antioxidant capacity.

ÖZET

ESMER PİRİNÇLE ZENGİNLEŞTİRİLMİŞ FONKSİYONEL YOĞURT ÜRETİMİ

Muhammad Usman AKRAM

Yüksek Lisans, Gıda Mühendisliği Bölümü

Tez Danışmanı: Prof. Dr. Ümran UYGUN

Ocak 2018, 62 sayfa

Bu çalışmada, çimlendirilmiş esmer pirinçten (GBR) elde edilen ham ekstraktlar kullanılarak γ-aminobütirik asit (GABA) içeriği yüksek fonksiyonel yoğurt üretilmiştir. Fonksiyonel bir yoğurt üretimi için, çimlendirilmiş esmer pirinç kullanılarak GABA zenginleştirmesi yapmak kolay, uygulanabilir ve ekonomik bir yöntemdir.

Esmer pirinç 96 saat süreyle çimlendirilmiş, kurutulmuş ve öğütülmüştür. GBR'de, çimlendirme periyodunun GABA ve toplam γ-oryzanol içeriğine ve antioksidan kapasiteye etkisi araştırılmıştır. GABA miktarının çimlendirme süresinin artmasıyla önemli oranda arttığı ve 84 saatlik çimlendirme sonunda başlangıçtaki miktarın yaklaşık 14 katına çıktığı gözlenmiştir. Buna karşın, γ-oryzanol'ün miktarında çimlendirmenin artmasıyla önemli bir değişim olmamıştır. GBR'ın toplam antioksidan kapasitesi, çimlendirmenin ilk 12 saatinde azalmış, 84 saatlik çimlendirmenin sonuna kadar küçük değişikliklerle artmıştır ($p < 0,05$).

Farklı düzeylerde GABA ile zenginleştirilmiş fonksiyonel yoğurtlar hazırlanmış ve 84 saatlik GBR ham ekstraklarının kullanıldığı üretim koşulları optimize edilmiştir. 20 ve 40 g un örneğinden hazırlanan ham GBR ekstraktları (13,88 ± 0,71 ve 26,86 ± 0,34 mg/100 g kuru madde GABA içeren), fonksiyonel yoğurt üretiminde kullanılmıştır. Depolamanın yoğurtlar üzerine etkisini belirlemede, saf GABA standardı ya da ham GBR ekstraktı katılan yoğurt örnekleri kontrol yoğurt örneği ile karşılaştırılmıştır. Kontrol yoğurdunda (CTR-Y) doğal oluşan GABA tespit edilmemiştir.

Fermantasyon sırasında, GABA standardı katılan yoğurt (CTR-G), 20 g ve 40 g GBR unundan elde edilen ham GABA ekstraktı katılan yoğurt örneklerinde (GBR20

ve GBR40), GABA içeriği sırasıyla %10,36, 14,39 ve 18,57 oranlarında azalmıştır ($p < 0,05$). Depolama süresince (21 gün) örneklerdeki GABA içeriğindeki değişiklik önemsiz bulunmuştur (GABA içeriği CTR-G yoğurt örneğinde 9,24 ± 0,07, GBR20 de 10,84 ± 0,14 ve GBR40 de 21,78 ± 0,13 mg/100 g). Kontrol yoğurdu ile karşılaştırıldığında, GABA içeren yoğurtların su tutma kapasitesinde (WHC), sertlik ve çiğnenebilirliği gibi tekstürel parametrelerinde 21 günlük depolama süresince önemli azalma olmuştur. Bununla birlikte, depolamanın ilk 14 gününde WHC ve tekstür, tüm yoğurt örneklerinde ilk artırılmış ve daha sonra, geri kalan depolama döneminde azalma gözlemlenmiştir. GABA içeren yoğurt örneklerinde yapışkanlık ve elastikiyet gibi tekstürel parametreler depolama ile iyi yönde gelişmiştir. Yoğurt örneklerinin toplam antioksidan kapasitesi ham ekstrakte edilmiş GABA ile arttırılmıştır fakat saf GABA eklenmiş yoğurtlarda toplam antioksidan kapasite, kontrol yoğurduna göre daha düşük bulunurken ($p < 0,05$), depolama süresi antioksidan kapasiteyi etkilememiştir.

Duyusal analiz sonuçları, GBR40 yoğurdunun kontrol yoğurduna göre kabul edilebilirliğinin düşük olduğunu, GBR20 yoğurdunun ise kontrol yoğurdunun yerini alabilecek uygunlukta olduğunu göstermiştir. GBR20 yoğurdunun bir porsiyonunda yer alan 10mg'dan yüksek GABA içeriği, literatüre göre yüksek tansiyonlu bireylerin tansiyonunu kontrol etmede yeterli bulunmaktadır.

Anahtar Kelimeler: γ-aminobütirik asit, çimlenmiş esmer pirinç'ın ham ekstraktı, fonksıyonal yoğurt, γ-orizanol ve toplam antioksidan kapasitesi.

ACKNOWLEDGEMENT

First and foremost, I would extend my heartiest gratitude to my supervisor, Prof. Dr. Ümran UYGUN, whose scientific expertise, constant encouragement, excellent guidance, constructive criticism, enlightened advice and moral support enabled me to accomplish my research goals. I learned a lot from her throughout the course of my study and research work. I thanked her very much for patiently correcting and editing my manuscript and for being generous and kind instructor to me.

I offer my sincere thanks to Presidency for Turks Abroad and Related Communities and Hacettepe University Scientific Research Projects Coordination Unit for awarding me a scholarship and research grant (Project No: FHD-2016-11950).

This research could not be possible without the kind support and technical guidance particularly in the area of product development of Late Prof. Dr. Yaşar Kemal ERDEM. Furthermore, I oblige to show my appreciations to Prof. Dr. Hamit KÖKSEL, Prof Dr. Dilek SİVRİ ÖZAY, Prof. Dr. Arzu BAŞMAN, Associate Prof. Dr. Ali TOPCU and Assistant Prof. Dr. Burçe ATAÇ MOGOL for their special guidance throughout my research work. I also owe my thanks to the technical experts Selin HEYBELİ, Meltem YILDIRIM, Yelda ZENCİR, and Hasan ŞİMŞEK for assisting me with their technical knowledge.

I would also like to share my gratitude to my department fellows (Tuğba BULAT, Ecem Evrim ÇELİK, Ezgi DOĞAN, Aytül HAMZALIOĞLU, Mithat KURBAN, Seda ELİKOĞLU, Yekbun URAS, and Azra Tuğce ÇINAR) and all my friends especially Dr. Muhammad Shazaib RAMAY, Noaman NASEER, Mohd MINHAJ, Muhammad ALI and Faisal HUSSNAIN for their generous help, cooperation, moral support and encouragement. I cannot finish without mentioning that how grateful I am with my family. I wish to thank my dear parents (Mr. and Mrs. AKRAM) and brothers. They have always supported and encouraged me to do my best in all of my education careers.

Muhammad Usman AKRAM

CONTENTS

Pages

LIST OF FIGURES

LIST OF TABLES

LIST OF ANNEX

SYMBOLS AND ABBREVIATIONS

Symbols

α	alpha
β	beta
γ	gamma
μ	mu (micro)
ρ	rho
ω	omega

Abbreviations

GBR	Germinated Brown Rice
RBO	Rice Bran Oil
GAD	Glutamate Decarboxylase
GABA	Gamma-Aminobutyric Acid
LAB	Lactic Acid Bacteria
AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemist
WHC	Water Holding Capacity
HPLC	High-Performance Liquid Chromatography
ABTS	2,2’-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid)

1. INTRODUCTION

In recent years, most of the consumers are changing their living standards with the consumption of functional and bioactive foods in order to live a healthy and natural life. Similar to traditional food, functional foods are consumed regularly as part of the normal diet. Apart from providing mere nutrition, these foods have positive effects on the health such as the reduction and prevention of chronic diseases [1]. The marketing shares and consumption of these types of foods are continuously increasing due to the reduction or prevention of disease risk. Functional foods also regulate specific physiological functions, such as delayed aging (age-related health problems) and strengthening of the immune system [2].

The dairy industry provides a huge opportunity for the development of a functional food market. The rapid growth of the global dairy industry in recent years has been attributed to the enhancement of functional properties in dairy products. In bioactive products, yogurt and fermented milk products are being preferred by the consumers for their healthy lifestyles. In recent years, more demand was recorded for the fermented milk products having natural ingredients.

In the United States, Canada, and China, functional yogurt market has grown very quickly, but same growth was not observed for the sales of probiotic/prebiotic yogurt in the European Union. Due to the high competition in functional food market, related food industries have been focusing on the development of the new formulation and innovation in the production of functional yogurt in order to increase the competitive strength. Various prebiotic components, barley, oats, rye, rice and wheat bran fibers are superior ingredients for the development of new formulations. And the functional properties gained in these products have also increased their commercial values [3].

Functional foods can be simple natural foods (such as tomatoes) or processed foods with enriched bioactive components. Enrichment of these components can be achieved by several means. Many types of foods (such as cereals, meat, dairy products and beverages) have been modified into functional products by biological transformation, for example, germination of brown rice can increase γ-aminobutyric acid (GABA) content as a bioactive component. Fermentation is another way of GABA enrichment by using GABA producing microorganisms. However, this

method for GABA enrichment may not be appropriate for each product. For example, it is better to add GABA in the form of germinated brown rice (GBR) for fortification of fermented products such as yogurt. There is a need for a simple and economical process to develop a functional product [4]. Fermentation for GABA enrichment is not suitable for Turkish type yogurt. As for yogurt production, only two bacterial species (*St. thermophilus* and *Lb. bulgaricus*) are allowed to be used, which have no GABA producing ability through fermentation.

In recent years, GBR has become an important functional food due to its rich GABA content. The use of GBR as an ingredient in bioactive food formulations has been identified as a common application in food industry, especially in the Far East countries. Since 1990, it is reported that numerous chronic illnesses (such as hypertension) can be prevented with the increased consumption of GBR [5].

In this study, the utilization of GBR as a bioactive ingredient have been investigated in the formulation of new functional yogurt. The production of GABA fortified functional yogurt at different levels was carried out and manufacturing conditions were optimized by using GBR flour and crude GBR extracts obtained from germinated brown rice flour.

2. LITERATURE REVIEW

2.1. Functional Foods

Normally, foods deliver nutrients which are important for body growth and proper functioning of its organs. It was documented that some types of foods known as functional foods, provide extra health benefits like prevention and treatment of several health disorders beyond the general nutrition effects. These foods can provide a healthy and natural way of life and also reduce the medicinal expenses [6]. The idea of functional foods development has changed the role of diets in health maintenance and disease prevention. Functional foods could be simple natural food or enriched with bioactive components. Conventional foods can be improved naturally or chemically by bioactive food components.

Recent studies have many pieces of evidence to support the beneficial role of functional foods and more investigations are being carried out on their health-promoting effects. Health issues like diabetes, hypertension, hypercholesterolemia, hyperglycemia and gastrointestinal problems are being taken as the targets of these functional foods [7, 8].

The dairy industry is an outstanding prospect for the development of functional food market. Enrichment of fermented dairy beverages with GABA and other inhibitory peptides is getting more attention due to their anti-hypertensive characteristic. Germinated brown rice is a valuable GABA source and used as functional ingredients. GBR also was known as a great source of other bioactive components like γ-oryzanol, polyphenols, phytic acid, ferulic acid, and tocopherols, which are well known for their health beneficial effects. Phenolic compounds as secondary metabolites of plants and γ-oryzanol are well-known strong antioxidant present in brown rice. Antioxidant effects are responsible for inhibition of several chronic disorder including diabetes, obesity, cancer, osteoporosis, atherosclerosis and cardiovascular diseases [9, 10].

2.2. Rice

Rice is an important cereal crop and considered a staple food for nearly half of the world's human population due to its high nutritional quality and digestibility.

Whole kernel grain (paddy rice) comprises of the outer protective layer (husk or hull) and internal edible rice fruit or caryopsis (Figure 2.1). After dehusking, brown or cargo rice are obtained, which consist of outer layers of bran (pericarp and embryo) and endosperm. Endosperm composed of internal starchy part and external aleurone layers, which also enclose embryo [11]. Rice grains are composed of 75-80%, starch, 7% protein and 12% water [12].

Complete polishing of paddy rice results in four fractions, which are hull, brown rice, bran and white rice. Rice bran and rice bran oil are important by-products obtained during polishing process in the production of white rice [13]. Because, rice germ and bran layers are a rich source of bioactive compounds (γ-aminobutyric acid and γ-oryzanol, tocotrienol, phytosterols and ferulic acid) along with the high amount of hypoallergenic protein, lipid, vitamin, mineral and dietary fibers [14]. Major phenolic compounds in dietary fibers of rice are ferulic acid and ρ-coumaric acids, primarily exist in free form, soluble conjugate form or insoluble bound form [15].

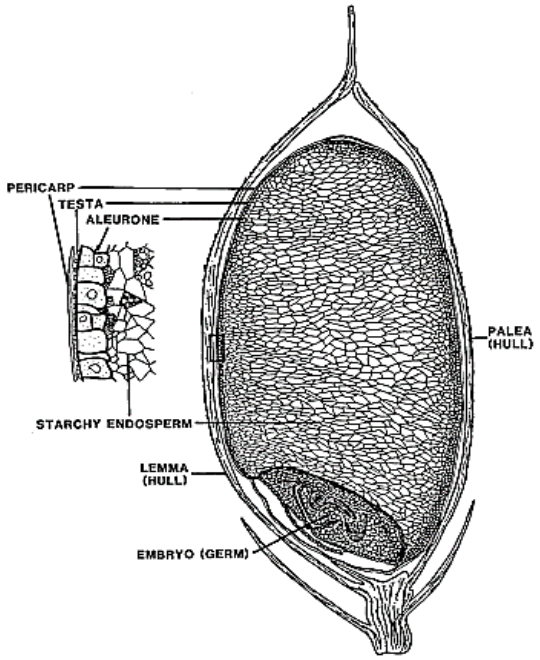


Figure 2.1. Structure of rice kernel [16].

2.2.1. Brown Rice

Brown rice contains many health-promoting components, but their taste acceptability and digestibility is lower than white polished rice and also more cooking time is required due to the high level of dietary fibers and phytic acid [11, 17]. However, consumption of brown rice is mostly preferred by health-conscious people of European countries [18].

Intake of white rice has been correlated positively with metabolic syndromes like diabetes type 2, glucose intolerance, obesity and cardiovascular diseases. In contrast, the consumption of brown rice has a therapeutic effect on the prevention of these diseases [19, 20].

Several studies in the literature indicated that quality, nutrition, texture, acceptability and phytochemical bioavailability of brown rice can be improved. And germination is an important economical process, which can be used to improve acceptability and phytochemical bioavailability of brown rice [10, 15]. GBR was established for marketing in Japan in 1995. Since then, GBR and its derived products have gained popularity in Japanese and Chinese markets, due to their improved nutritional attributes [21]. Subsequently, GBR as functional ingredient came to the attention of food scientists and there is a rising trend for using GBR in the formulation of superior quality foods.

2.3. Germinated Brown Rice (GBR)

Plants have the ability to store food reserves (starchy carbohydrate, protein, fat etc.) in seeds, which are utilized for the growth of embryo during germination by sufficient absorption of water (imbibition), and result in disruption of the seed coat. Germination mostly depends upon both external and internal environmental conditions. Favorable conditions are adequate water supply, suitable gas atmosphere, temperature and light availability, which differ with seed types and varieties. The most suitable atmosphere for seed germination is the presence of 20% O_2 and 0.03% CO_2 gases [22].

GBR is produced by water soaking of brown rice kernels at 30-40°C until embryos start to sprout [23]. Germination is a biological process that drastically effects chemical composition of rice. During this process, subcellular structures changes, respiration, macromolecular syntheses and cell elongation have occurred in mature dry seeds [24].

During germination of seed, water was absorbed in three different phases as shown in Figure 2.2**,** where the curve is a representation of time course for water absorption. The physiological activities start within a few minutes of initial absorption of water in phase 1. During phase 2 (plateau phase), the water content in seeds remains unchanged and metabolic activities accelerate with the consequential

transcription of new genes. At the end of this phase, the appearance of radicle by surrounding structures reveals the termination of germination, and in phase 3 further water uptake takes place through utilization of stored reserves by young seedling [25]. The time required to complete these events fluctuates for different seed species and germination conditions.

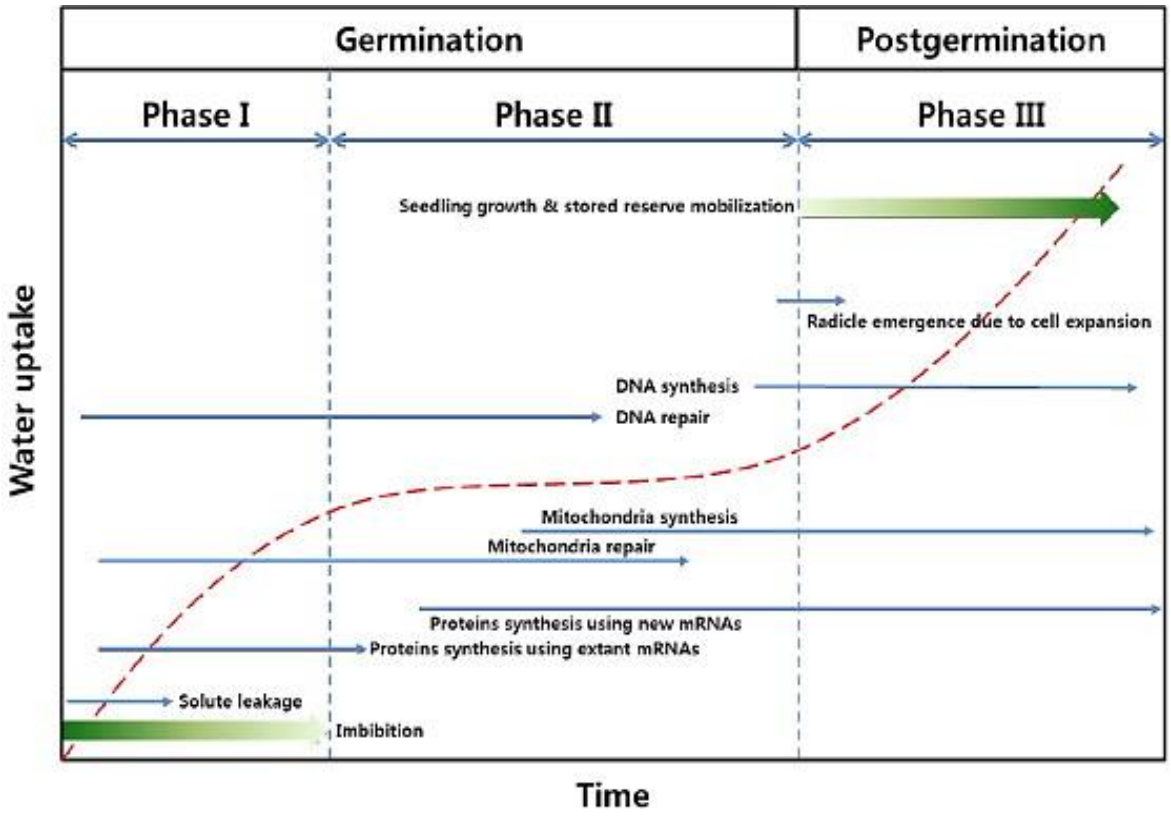
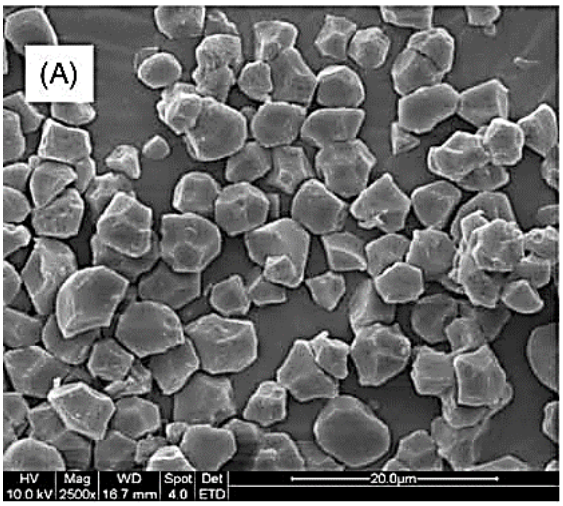


Figure 2.2. Time progress of major steps involved in germination and post-germination growth [26, 27].

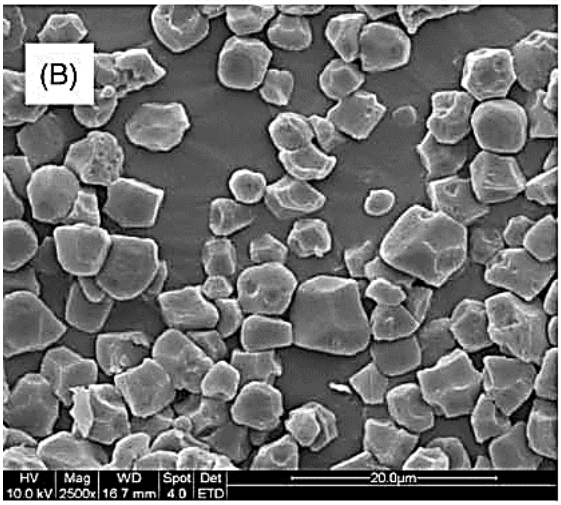
This water uptake results in activation of hydrolytic enzymes. These enzymes degrade complex high molecular substances (starch and proteins) into small molecules (simple sugars, peptides, and amino acids). It was reported that starch and associated proteins degraded by the action of amylases and proteases. As in Figure 2.3**,** starch granules of brown rice have polyhedral and irregular in shapes with a size of 3 to 8 μm (A), but after germination, they became smaller and more diverse (B) because of the partial degradation of starch by hydrolytic enzymes [27]. Further water uptake results in disruption of seed membranes and consequently leakage of low molecular weight substances into the surrounding solution.

Degradation of high molecular weight polymers leads to improved organoleptic qualities with the production of various bioactive and flavor compounds along with

germination period [28]. Also, germination process improves the bioavailability of nutrients by neutralizing the phytic acid. During sprouting, a reaction between phytic acid and minerals occurred, which result in a soft texture, fast cooking and easy digestion of GBR. Sensory evaluation revealed that cooked GBR are more sweet, soft and swelled than regular cooked brown rice [29, 30].



C: BR (×2500)



E: GBR (×2500)

Figure 2.3. Scanning electron micrograph for starch of brown rice (BR) and germinated brown rice (GBR) [31].

During germination, accumulation of bioactive components in brown rice such as γ-aminobutyric acid (GABA), γ-oryzanol, phytosterols and phenolic compounds, greatly depends upon the cultivar type, additives presences, pH, temperature, time, light, and oxygen percentage of soaking water [20]. GBR have nearly 10 times more GABA, 4 times more dietary fibers, lysine, vitamin E and B3, and 3 times more vitamin B1 and B6, as compared to polished white rice [32].

Germinated brown rice extract having GABA content can inhibit proliferation of leukemia cells along with stimulatory effects on the immune cells and apoptosis of cancerous cells [33].

2.3.1. γ-aminobutyric Acids (GABA)

GABA is biogenic compound and consist of four carbons non-protein amino acid, where amino group exists in unbound form. It is a major inhibitory neurotransmitter found in the central nervous system (CNS) of mammals. At physiological pH values of 4.03 and 10.56, GABA is zwitterionic in nature because of carrying both positive and negative charges (Figure 2.4). It is highly water soluble and structurally flexible molecule, with numerous configurations in solution form (including cyclic structure, similar to proline) [34].

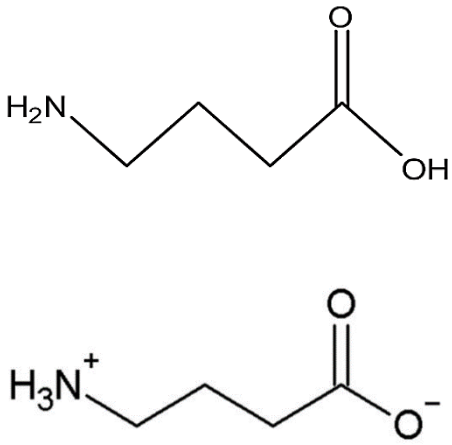


Figure 2.4. Structure of GABA (simple and zwitterionic form) [27, 35].

It is irreversibly produced by α-decarboxylation of L-glutamic acid or its salt, catalyzed by glutamate decarboxylase (GAD; EC 4.1.1.15) enzyme and its cofactor pyridoxal-5-phosphate (P5P) [36, 37]. In the plant, GABA influenced various processes such as storage of nitrogen, utilization of glutamic acid, plant growth and defensive system against various insects [38]. The natural formation of GABA was first described by Steward et al. [39] in potatoes roots. However first time accumulation of large GABA amount in rice grains was reported by Saikusa et al. [40], who observed an increase in GABA content under soaking in acidic conditions.

Germinating conditions like soaking and sprouting at 30°C for 3 and 21 hours respectively, were reported to be optimum for highest GABA production in brown rice [30]. In another report, soaking of brown rice in a solution of pH 6.0 for 4 hours at the temperature of 40°C, was said to be optimum for high GABA production [27].

At an early stage of germination, rapid GABA production occurred by the parallel loss of glutamic acid or its salt [14]. It is due to the activation of the enzyme (glutamic acid decarboxylase), which controls GABA synthesis from glutamate or glutamic acid. Soaking may cause conditions of hypoxia, and GABA content increases as a result of low oxygen availability [41].

An increased concentration of GABA has been reported in brown rice, germinated in a solution of glutamic acid alone or mixture of chitosan and glutamic acid [33]. The use of electrolyzed oxidizing water increased GABA content during germination, with shortened sprouts [42]. GABA production can be enhanced by environmental stress due to mechanical and environmental stimulations, like heat shock, cold shock, hypoxia, darkness and cytosolic acidification [27].

In recent years, GABA producing lactic acid bacteria (LAB) are also getting huge attention due to their GAD enzyme activities [43]. GABA fortified fermented milk was manufactured by using *Lactobacillus casei* strain Shirota and *Lactococcus lactis* YIT 2027 [44]. Where milk protein (casein) was first hydrolyzed into L-glutamate by *Lb. casei* and then it is converted into GABA by *Lc. lactis*. Similarly, defatted rice bran extract was fermented by two Lactobacillus species (*Lb. brevis* and *Lb. plantarum*) and GABA synthesis was achieved without the addition of glutamate. And after 24 hours of fermentation, 2,952 ppm of GABA was accumulated by *Lb. brevis* at pH 5.0 [45].

GABA has many health beneficial effects like antidepressant [46], antihypertensive [47], antiepileptic and antidiabetic effects [48]. It also involved in regulatory functions of cardiovascular systems like heart rate and blood pressure, and responsible for the sensation of pain and anxiety. Metabolism in brain cell is promoted by GABA because of its ability to increase the supply of oxygen [49]. GABA can also prevent obesity by ameliorating the oxidative stress and high fatty diet disrupted functions of thyroid hormones [50]. In the human brain, GAD substrate (L-glutamate) acts as an excitatory neurotransmitter and its product (GABA) as an inhibitory neurotransmitter. Therefore, control of GAD and GABA levels was observed to prevent several neurological disorders like Huntington’s disease, Parkinson’s disease, Alzheimer’s disease, schizophrenia, seizures, dementia and stiff-man syndrome [37, 38]. The consumption of GABA along with foods has been scientifically proved to lower the systolic blood pressure elevation and also improve

learning abilities and memory in animals. The proliferation of cancerous cells can be depressed or blocked by using GABA containing germinated brown rice extract [33]. In one study, it was reported that GABA enriched fermented milk has higher angiotensin-1-converting enzyme (ACE) inhibitory activity than control milk [51]. Therefore, hypertension, high blood pressure and resulting cardiovascular diseases can be prevented by consuming GABA containing fermented milk [52, 53].

GABA-enriched foods are therefore seen as functional foods and have become popular for the alleviation of pain and anxiety, and can cure insomnia and chronic alcohol-related symptoms [38, 54]. Japanese people have already started to use purified GABA in medication treatments of brain blood channels [14]. For these reasons, GABA as a new type of functional food supplement has been widely used in food, pharmaceutical, and chemical industry.

2.3.2. γ-oryzanol

First isolation of γ-oryzanol was accomplished in 1954 by Kaneko and Tsuchiya [55] as a crystalline substance from the non-saponifiable fraction of rice bran. It was believed to be a single component and was named as oryzanol in reference to the botanical species of rice, *Oryza sativa*. Later more than twenty components of γ-oryzanol as ferulic acid esters of sterols and triterpene alcohols have been identified by different extraction methods. γ-oryzanol is a mixture of structurally similar components particularly steryl ferulates. Steryl ferulates are esters of ferulic acid with triterpene alcohols and phytosterols (Figure 2.5). As the major components, campestanyl ferulate, cycloartenyl ferulate, and 24-methylenecycloartanlyl ferulate constitute nearly 90% of GBR's γ-oryzanol [27].

γ-oryzanol mainly present in rice bran oil (RBO) as a non-saponifiable fraction. It has a great antioxidant capacity and therefore has several health-promoting effects like anti-aging, anti-inflammation, anti-platelet aggregation and cholesterol lowering effects [56]. Antioxidant capacity of brown rice is mostly attributed to ferulate part of γ-oryzanol. Due to the oxidation of cholesterol by γ-oryzanol, these antioxidant properties are also associated with hypocholesterolemic effects [27]. Similarly, ferulic acid as a major phenolic component of oryzanol has displayed significant preventive behavior against aggregation of blood platelets and formation of superoxide [57].

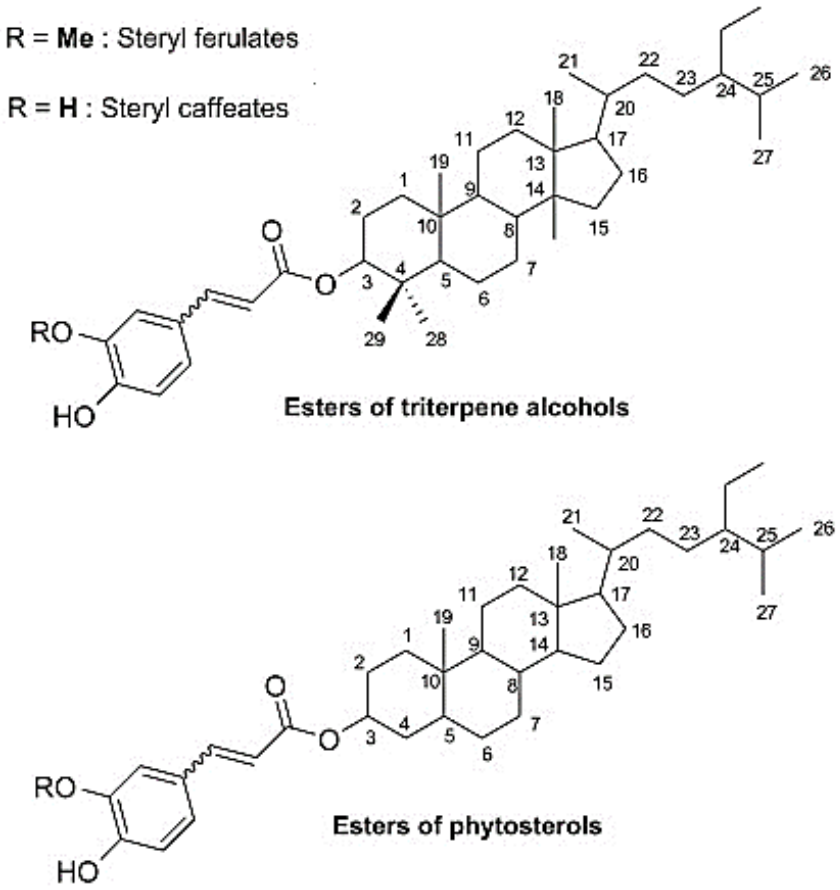


Figure 2.5. Components of γ-oryzanol [58].

2.4. Yogurt

Yogurt is a fermented dairy product, which is manufactured by the bacterial fermentation of milk. Fermentation is a metabolic process in which various chemical changes are carried out on organic substrates through the actions of microbial enzymes [59].

Turkish standard yogurt is manufactured by fermenting milk with a symbiotic culture of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (in 1:1 chain ratio). Culture bacteria should be active, viable and sufficient in number (at least 10^6 CFU/mL) up to 28 days shelf life of yogurt [60]. Symbiotic metabolism of these two bacterial species has positive combined effects on the fermentation properties (population growth and rate of lactic acid production) as compared to the metabolism of a single culture. In this type of symbiotic relationship, one bacterium produces substances, which are necessary for the growth of other bacterium. For instance, *St. thermophilus* create acidic and

anaerobic conditions by the production of formic acid, lactic acid and carbon dioxide, which supports the multiplication of *Lb. bulgaricus*. In return, *Lb. bulgaricus* stimulate the growth of *St. thermophilus* by the production of histidine, valine, glycine, glutamic acid, leucine, isoleucine and short peptides [61].

Generally, yogurt can be made by various types of milk, but cow's milk is mostly used for the production of industrial yogurt. Milk should be free from antibiotics and bacteriophages contamination and have a low microbial count for the better growht of yogurt culture [62]. Typical whole fat yogurt has lactic acid titratable acidity ≥0.9% with milk fat 3.25% and milk solids not fat (MSNF) 8.25% [63]. Standard yogurt should have 13% total solids (TS), as the optimum growth of *Lb. bulgaricus* and *St. thermophilus* occurs in 12 and 14% TS, respectively [64]. Many techniques including boiling, evaporation, membrane filtration and addition of milk or whey powder are used for TS increase [65]

On the base of production, yogurt is further divided into the set and stirred type yogurt. Set type yogurt is made by culturing the milk by the LAB in consumer containers and gel structure of curd is protected until the consumption. While for manufacturing of stirred type yogurt, first yogurt gel is obtained in large tanks, and curd is broken by stirring or agitation, then smooth and viscous yogurt is packed in cups for marketing. Both types of yogurt are further divided into several other commercial categories such as natural/plain yogurt, fruity yogurt, flavored yogurt and functional yogurt etc. [66].

2.4.1. Yogurt Processing

The manufacturing process for set type yogurt (Figure 2.6) involves various processing steps like standardization, homogenization, pasteurization, cooling to incubation temperature, inoculation of LAB starter culture, packaging, incubation for fermentation (till pH 4.60), cooling and storage (4°C), which irreversibly modifies properties of milk [67].

In the dairy industry, standardization of milk is necessary in order to achieve the same quality of yogurt during production. Total solids (fat and MSNF) are standardized either by removal or addition of desired substances. Generally, commercially manufactured yogurt has total solids in the range of 14-15%. Where total solids were increased by evaporation under vacuum treatment or manual

addition of skim milk powder. Stabilizers (pectin or gelatin) are also used for the improvement of texture and mouth feel [66].

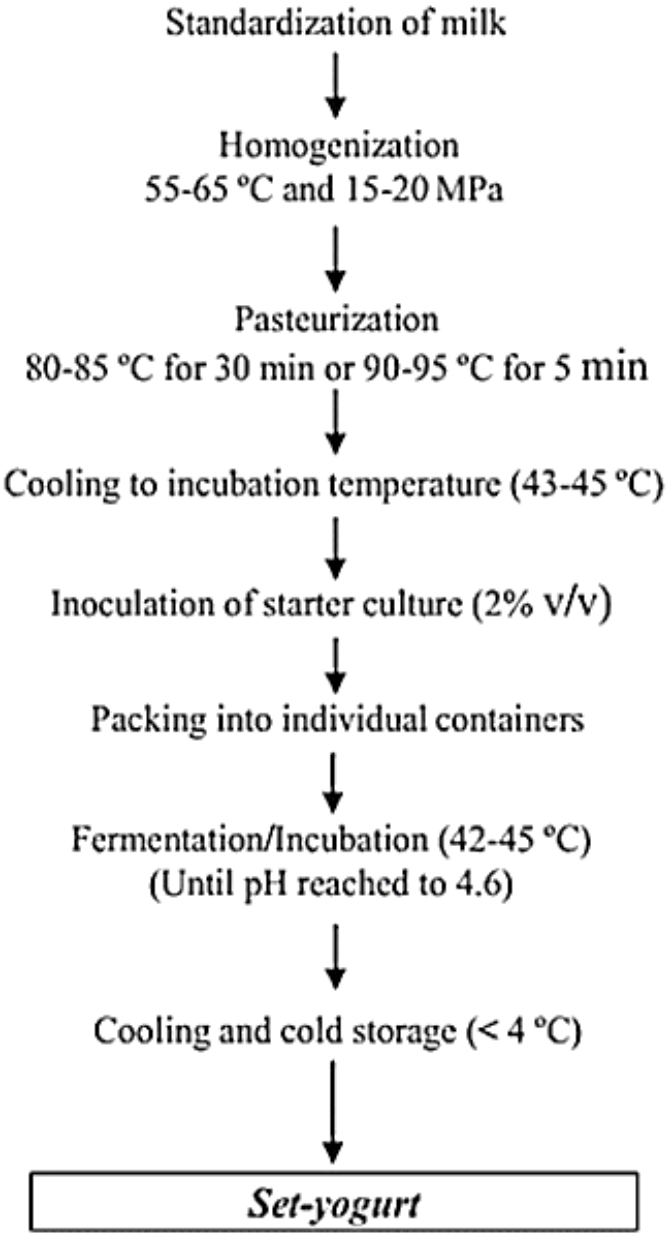


Figure 2.6. Main processing steps in the manufacturing of set type yogurt [67].

Homogenization of milk is carried out by the high-pressure homogenizer at 15-20 MPa pressure and 60-70°C temperature [68]. It is used to minimize fat separation from milk by gravity. It also produces white and viscous yogurt with homogenously distributed flavor [69]. After homogenization, the surface area of milk fat globules increases, therefore, it modifies the interactions with protein particles. Mostly, milk is homogenized before heat treatment in order to avoid the problem of post contamination in homogenizer [70].

The basic purpose of the heat treatment is to kill pathogenic microorganisms in raw milk. But for yogurt manufacturing, milk is processed above the normal pasteurization temperature (72°C for 15 sec). This improves the texture and water holding capacity of yogurt due to the denaturation of whey protein (β-globulins) on the surface of κ-casein. In yogurt production, two type of time-temperature treatments can be applied for pasteurization. One is known as batch pasteurization (80-85°C for 30 min) and other is continuous pasteurization (90-95°C for 5 min) [66]. Yogurt culture is oxygen sensitive and so heat treatment results in the removal of dissolved oxygen, hence it assists the growth of culture.

After pasteurization, milk is cool down to incubation temperature (42-43°C) and LAB mix culture (*St. thermophilus* and *Lb. bulgaricus*) is used for the inoculation at a chain ratio of 1:1. And milk samples were incubated at an optimum temperature of 43°C for about 4 hours. Generally, fermentation time depends upon the rate of inoculation and temperature of incubation. When pH of yogurt samples reduces to 4.60, fermentation is rapidly stopped by cooling.

There are two methods of cooling used in yogurt production; in one phase cooling, yogurt samples are cold to 5°C after fermentation and stored. While in two-phase cooling, temperature first reduced to 37°C then to 10°C, and finally stored at 4°C until the consumption [65].

2.4.2. Chemistry of Yogurt Production

Degradation of milk components by LAB cultures results in the production of lactic acid and many other metabolites like peptides and organic acids. Some of them have health beneficial effects along with improved textural characteristics for fermented products like yogurt and cheeses [51].

In yogurt manufacturing, bacterial culture degrades lactose (disaccharide sugar) into two monosaccharides; glucose and galactose with the action of β-galactosidase enzyme, yet at the end of the fermentation process, lactose percentage is higher than other monosaccharide. Fermentation reduced lactose contents from 4.8 to 2.5-2.6%. A complete breakdown of total lactose is not reachable; as bacterial growth declined with continuous production of lactic acid. Still, this residual lactose does not show any discomfort to lactose intolerant people [70].

The acidification process by fermentation of lactose to lactic acid results in solubilization of colloidal calcium phosphate (CCP) and it also disrupts the structure of casein micelles [71]. When pH of milk drops from 6.7 to 5.0, the net negative charge of casein micelles reduced with shrinking of charged hairy sites on κ-casein. It also decreases steric stabilization and electrostatic repulsions among micelles [72]. In addition, this fall in pH causes a decline in ionization and surface potential of the acidic groups in casein protein (aspartic, glutamic and phosphoserine residues). Ultimately, progressive removal of calcium (Ca) and inorganic phosphates (PO_4^{-3}) from casein micelles to aqueous phase take place. In milk, complete solubilization of CCP occurred at pH ~5.0 [73]. When pH of milk reduced from 5.0 to 4.6 (isoelectric point of casein), increased hydrophobic and electrostatic interactions result in the formation of three-dimensional gel (acid curd) [74].

2.4.3. Flavor and Aroma

Flavor and aroma development is the most important property for the sensory evaluation of fermented products. Sensory properties in dairy products mainly depend upon the relative balance of flavor and aromatic compounds derived from milk components (carbohydrate, protein or fat etc.).

Aroma and flavor of yogurt are generally developed by fermentation process due to the production of volatile, non-volatile and carbonyl compounds. Source of milk and type of starter culture are two main responsible factors for the organoleptic properties. Typical flavor of yogurt is imparted by lactic acid and mixture of aromatic compounds. More than 90 volatile flavor compounds have been identified, among them acetaldehyde, diacetyl, acetoin, acetone, and 2-butanone play a significant role in bringing the typical aroma in yogurt [75].

Acetaldehyde is the major aroma compound in yogurt, which delivers characteristic green apple or nutty flavor to yogurt. The production of acetaldehyde is predominantly achieved by LAB without alcohol dehydrogenase enzyme. Also, a significant increase in the level of acetaldehyde was observed in the symbiotic growth of *St. thermophilus* and *Lb. bulgaricus*.

On the other hand, diacetyl improves yogurt quality at high concentration by supplying buttery flavor. It is produced by fermentation of citrate in milk. At the ratio of 1:1, acetaldehyde and diacetyl can provide a preferred distinct flavor to yogurt.

The conversion of diacetyl into acetoin occurred by enzyme diacetyl reductase. Similar to diacetyl, acetoin also has mild-creamy and sweet butter like flavor. Whereas, Acetone and 2-butanone impart sweet and fruity aromas with a minor contribution in yogurt flavor [76].

2.4.4. Texture

The texture is considered as one of the essential features for yogurt quality. In food products, texture explains all rheological and structural attributes achieved by the means of mechanical, visual and sensorial evaluation [77].

Texture evaluations are either subjective by trained panelists (sensory evaluation) or objective using an instrument (mechanical or rheological study). Instrumental measurements of texture profile are achieved by using two-stage compression test in texture profile analyzer (TPA) by probes of different shapes. Force (stress) and deformation (strain) are two major factors of texture characterization [78]. A number of textural parameters such as hardness, gumminess, cohesiveness, and springiness can be determined from resulting force-time curve, also known as TPA curve [79].

Hard structure, aggregation, and serum separation are considered as most important examples of textural impairments. As low total solids, insufficient heat treatments, low acidity or high incubation temperature are responsible for the poor texture of yogurt. Also, reduction of fat can result in the development of fragile texture due to weak protein gel formation in yogurt [80].

Yogurt is classified as pseudo-plastic material that can be either viscoelastic solids (set type) or viscoelastic fluid (stirred type). Viscoelastic means that material exhibit both elastic and viscous properties under deformation. Structural breakdown of yogurt by applied shear force is not reversible, even after removal of stress [74].

Several parameters like quality and composition of milk, time/temperature combination for heat treatment, starter culture type or quantity, incubation and storage temperatures have major effects on textural properties of yogurt [81]. Yogurt texture can be improved by denaturation of milk proteins with heat treatment above 70°C, that also improve water holding capacity. Moreover, a little increase in TS positively affects the texture and viscosity of yogurt [82]. But very high TS negatively affect the propagation of LAB culture due to less remaining water.

Water holding capacity (WHC) is another way to express the strength and firmness of yogurt. It can be defined as the percentage of the concentrated gel obtained after static or centrifugal drainage. As a forced syneresis, WHC does not provide any information about spontaneous syneresis defect in set type yogurt [83].

2.5. Functional Yogurt

Yogurt is considered as a vehicle for delivering bioactive peptides and probiotic bacteria. It also has numerous health benefits like lactose intolerance, bone mineralization, weight management, laxation and gut-associated immune response [84]. These health beneficial effects can be improved by the addition of probiotic bacteria and bioactive peptides in conventional yogurt and their control release in human gastrointestinal tract through proteolytic activities. Due to the presence of prebiotics and probiotic bacteria, *Activia*® type functional yogurts can also recover digestive health [62]. Also intestinal functions can be favorably modified by the consumption of *Activia*® yogurt containing *Bifidobacterium animalis* (10^8 CFU/g) [85].

The most important type of functional yogurt is probiotic yogurt, also known as bio yogurt. It consists of additional starter cultures (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*, also known as AB culture), that play an important role in modification of human's gut because of their abilities to survive in intestinal tract [86]. Fruits based dietary fibers (as prebiotics) were also used for the production of probiotic yogurt. Their effects on titratable acidity and fatty acid profiles of skim milk probiotic yogurt types were studied. And it was reported that fatty acid profile of functional yogurt can be improved by fruits based dietary fibers [87]. Probiotic yogurt with passion fruit peel powder was prepared and it was observed that peel powered caused reduction of fermentation time and the rise of titratable acidity with texture improvement [80, 88]. In another study textural firmness was significantly affected during the preparation of probiotic yogurt by concomitant supplementation of vegetal oil emulsion and passion fruit peel powder, with no influence on fermentation time [89]. However, the addition of pineapple peel powder is reported to adversely affect both fermentation time and firmness of probiotic yogurt [90].

In functional yogurt, the addition of flaxseed and blackcurrant oils were accomplished in order to increase the level of ω-3 fatty acids (α-linolenic acid). And

this addition did not cause any effect on the growth of LAB [91]. Also, Rognlien et al. [92] used butter and fish oils for the enrichment of ω-3 lipids in functional yogurt and the effect of their oxidation was observed on the sensory perceptions. And the flavor and overall acceptability were slightly acceptable for yogurt samples with butter and fish oil.

On the other hand, yogurt fortification with encapsulated polyphenols of olive fruits enhanced the digestion and lipid metabolism by promoting the bacterial growth of LAB during fermentation and storage. It also revealed an adverse effect on the growth of spoilage microorganisms (yeast and molds etc.). Additionally, reduction of body weight, blood pressure, and LDL (Low-density lipoprotein) cholesterol level have been reported with the consumption of olive-based yogurt for 2 weeks ($p < 0.05$) [93]. During initial incubation, pH was rapidly dropped to 4.60 in yogurt samples enriched with olive polyphenols and then during storage protective effects against undesirable pH reduction was contributed by these polyphenols (in the concentration of 500-1000 ppm) [94]. In another study, high levels of phenolic and anthocyanin compounds (78.46 and 17.7 mg/kg, respectively) were reported in yogurt samples prepared from red grape extracts. Radical scavenging capacities of these yogurt samples decreased with storage time but no significant difference for aroma was observed by sensory evaluation [95].

In recent years, resveratrol (trans-3 5 4'-trihydroxystilbene) has received considerable attention due to its anti-proliferative effects (chemo-preventive) on cancerous cells. In a study, Emirdağı [96] used 100 ppm standard resveratrol for yogurt production, and about 85% resveratrol was found to be attached to milk proteins even at the end of 21 days of storage. Also, it has been reported that resveratrol has no adverse effect on the production and texture of yogurt during fermentation and storage period.

2.5.1. GABA Fortified Yogurt

A number of studies have reported manufacturing of GABA fortified yogurt by supplementation of germinated brown rice. GABA fortified yogurt was developed by using LAB and GBR with a higher concentration of GABA (137.17 µg/g of dry matter) [97]. Also, soy yogurt with GABA content of 424.67 µg/g (dry matter) was prepared by using germinated soybean extracts and LAB [98]. In both cases, fermentation

was achieved for 24 hours by using high GABA producing LAB species (*Lactobacillus acidophilus*, *Lactobacillus plantarum,* and *Lactobacillus brevis* OPY-1) at 37°C.

On the other hand, some studies developed GABA enriched yogurt by using conventional yogurt culture and GBR. Generally, GABA containing GBR (in flour or paste form) were mixed with skim milk powder and fermentation was achieved for 12 hours at 37°C after pasteurization [99, 100]. Whereas, Liu et al. [101] used bovine raw milk and pure GABA standards for the manufacturing of fortified yogurt and described that GABA addition (0.5%) has a great role on the improvement of textural properties in yogurt.

In another study, *Lb. plantarum* NDC75017 along with commercial starter culture was used as GABA producing LAB in skim milk and fermentation was accomplished for 48 hours at 30°C. Higher GABA quantity of 314.56 mg/100 g was produced after optimizing the concentration of coenzyme P5P (pyridoxal-5-phosphate at 18 µM) and MSG substrate (L-monosodium glutamate at 80 mM) [36]. Similarly, GABA producing *St. thermophilus* APC151 was used for yogurt manufacturing from the skim milk having 2.25 mg/mL MSG. And after fermentation of 48 hours at 42°C, GABA was produced in the amount of 2.10 ± 0.16 mg/mL coagulated milk [102].

3. MATERIAL AND METHODS

3.1. Materials and Chemicals

Brown rice (*Japonica* varieties of *Oryza sativa L.*) was purchased from the local market (Migros, Ankara). The fresh whole cow milk was supplied from A.O.Ç (Atatürk Orman Çiftliği) Dairy Industry, Ankara and brought to the laboratory of Department of Food Engineering, Hacettepe University, on the same day in cold storage conditions.

Whereas, HPLC grade methanol, acetonitrile, ethanol, γ-aminobutyric acid standard, γ-oryzanol standard, C18 Supelcosil LC-DABS (4.6 mm i.d. ×150 mm) column, dabsyl chloride, ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], potassium persulfate, Trolox standard, sodium hydroxide, hydrochloric acid and dihydrogen potassium phosphate were purchased from Sigma-Aldrich (Germany).

3.2. Germination of Brown Rice

Brown rice was cleaned with sufficient amount of distilled water. For germination of brown rice, cleaned brown rice was soaked in distilled water (ratio 1:3, w/v) and placed in an oven at a temperature of 35°C. The water was replaced by equal quantity after every 4 hours' interval [103]. At the end of 0, 12, 24, 36, 48, 60, 72, 84 and 96 hours of germination, samples were oven dried at 30°C, milled by coffee grinder (Tefal, Ankara) for 3 min and stored in closed plastic containers at 4°C for further analysis.

3.3. Compositional Analyses of Rice Samples

The total solids, fat, protein and ash contents of rice samples were determined according to the methods of American Association of Cereal Chemists (AACC) [104].

3.4. GABA Extraction from GBR

Extraction of GABA from 84 hours GBR was achieved according to Soi-ampornkul et al. [105], with some modifications (Figure 3.1). Briefly, extraction of dried GBR flour was obtained with 2-steps rinsing by deionized water (20 and 10 mL) and centrifugation (Sigma 3-18K, Germany) at 5000 and 8000×g for 20 and 10 min respectively, at 20°C. This method was repeated for two and four times, in order to

get crude extracts from 20 and 40 g GBR, respectively. All supernatants were collected after passing through 1 mm pore sized Whatman filters, and autoclaved at 121°C and 15 psi for 15 min. Finally, sterilized supernatants were lyophilized by freeze dryer and used for the production of functional yogurt.

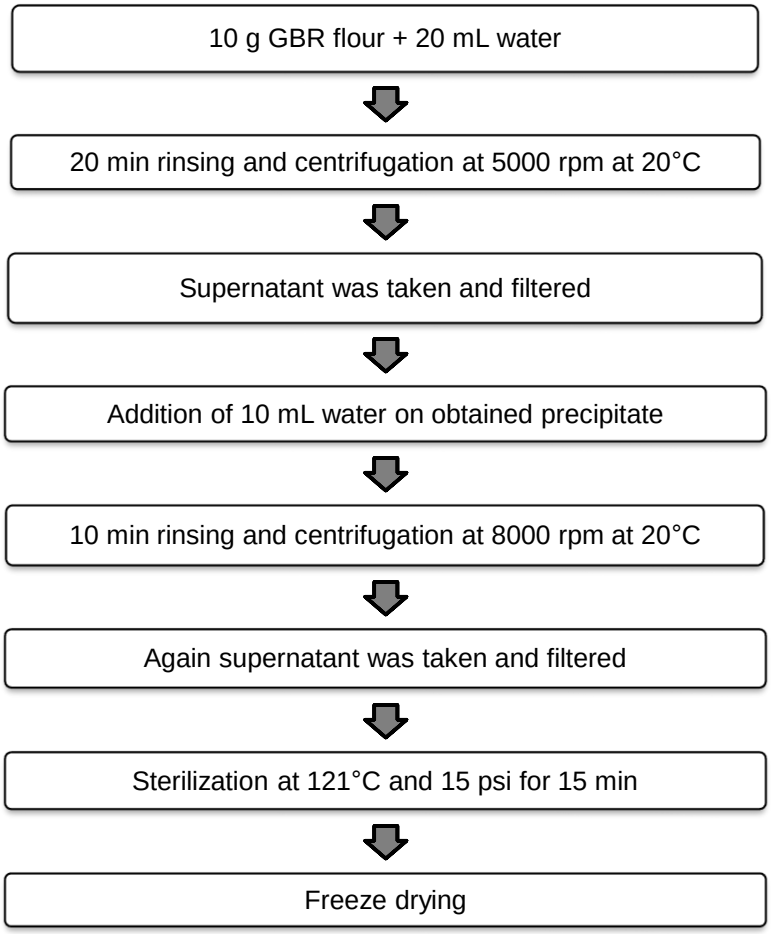


Figure 3.1 Preparation of crude GBR extracts.

3.5. Production of Control and GABA Fortified Yogurt

Control and GABA fortified yogurt were prepared according to Liu et al. [101], with minor modifications. For the production of control yogurt, milk was pasteurized at the temperature of 95°C for 5 min in order to destroy pathogens and then cooled to 43°C. Inoculation was achieved with conventional yogurt culture *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. And mixture of pasteurized milk and LAB culture was incubated at 43°C for 3-4 hours. Yogurt fermentation was sustained till pH value of 4.60 and then stopped by rapid cooling to 4°C.

For the production of GABA fortified yogurt, initial steps were the same as described above. Brown rice after 84 hours of germination were selected for extraction

because of higher GABA content. After sterilizing and freeze-drying, GABA containing crude GBR extracts were mixed with pre-pasteurized milk samples (100 g). Similarly, pure GABA standard (10 mg) was also added into milk (100 g). And all milk samples with GABA were homogenized at 15000 rpm for 3 minutes. After homogenization (60°C), cooling, and the addition of LAB culture, fermentation was achieved at 43°C. At the end of fermentation, all types of yogurt samples were stored for 21 days. Detailed information about the differences among the all types of yogurt samples is given in Table 3.1.

Table 3.1. The differences among all types of yogurt samples.

Abbreviation	**Yogurt Samples**
CTR-Y	Control Yogurt without any addition
CTR-G	Yogurt with 10 mg GABA standard for 100 g milk
GBR20	Yogurt with crude extracts of 20 g GBR for 100 g milk
GBR40	Yogurt with crude extracts of 40 g GBR for 100 g milk

For all yogurt samples, physicochemical analyses such as compositional analyses, WHC, texture, GABA content and total antioxidant capacity were determined at intervals of 1, 7, 14 and 21 days of storage. However, sensory evaluation was performed only for CTR-Y, GBR20, and GBR40 types yogurt samples after 1 day of storage.

3.6. Physico-chemical Analyses of Milk and Yogurt

The pH of milk and yogurt samples was measured by hand type pH meter (EZ Do 7011, Gondo Electronic Co. Ltd., Taiwan). Whereas the titratable acidity (% lactic acid), total solids, fat, protein and ash contents in milk and yogurt samples were determined with reference of Association of Official Analytical Chemist (AOAC) [106].

3.7. GABA Analyses for Rice and Yogurt

About 0.25 g GBR and 1 g yogurt samples were placed in Eppendorf tube containing 1 mL of 70% ethanol and vortexed for 1 minutes. Then the mixture was centrifuged (Sigma 3-18K, Germany) at 7000 rpm (4°C) for 10 minutes and 300 µL supernatant was removed. After adding 50 µL 0.1 N HCl and 450 µL 0.15 M $NaHCO_3$ (pH 9.2)

on the removed supernatant, it was stirred for 1 minute. For derivatization, 100 µL of 4 mM dabsyl chloride (prepared in acetonitrile) was added and mixed well then placed in water bath at 70°C for 15 minutes and the reaction was stopped by sudden cooling. A mixture of 70% ethanol and 25 mM KH_2PO_4 was prepared in 1:1 ratio, then 500 µL of it, discharged into the previous mixture and centrifuged at 15000×g for 5 minutes at 10°C. After passing through 0.22 µm syringe filter (Millipore, Bedford, MA, USA), the supernatant was taken into HPLC vials [107, 108].

GABA standards were prepared similarly by using a different amount of pure GABA (0, 5, 10, 20, 40 and 80 mg/L) in 70% ethyl alcohol and calibrated curve and correlation constant were determined. The obtained calibration curve and correlation coefficient (R^2 = 0.9935) are shown in Annex 1. Whereas HPLC chromatograph for 40 mg/L standard GABA is shown in Annex 2.

For analyses, Agilent Technologies 1100 HPLC (Waldbronn, Germany) containing binary pumps system, diode array detector (DAD) and thermostat column were used. Injection volume was 5 µL and mobile phase composition was A: 25 mM, KH_2PO_4 (pH 6.8) and B: acetonitrile/methanol (70:30%) with a flow rate of 1.5 mL/min and mobile phase gradient: (Time: B%): 0-9 min: 20%; 9-23 min: 20-35%; 23-24 min: 35-40%; 24-30 min: 40-45%. Chromatographic separation of dabsyl amino acid (dabsyl-aa) was carried out by using C18 Supelcosil LC-DABS (4.6 mm i.d. × 150 mm) column and DAD wavelength of 456 nm. The total analysis was completed in 40 min. Duplicates were analyzed independently and results were described in mg GABA/100 g of rice (on dry basis) and yogurt sample (on fresh basis).

3.8. Total γ-oryzanol contents

Total γ-oryzanol contents were determined by using HPLC, according to Jeng et al. [109], with little modifications. About 1g rice sample was mixed with 4 mL methanol. After vortexing for 1 minute, it was subjected to centrifugation for 10 minutes at 850×g (Sigma 3-18K, Germany). Then, after filtration through 0.22 µm syringe type filters (Millipore, Bedford, MA, USA), the supernatant was taken into HPLC vials and stored at -20°C for HPLC analysis (Agilent 1100, series, Waldbronn, Germany).

For calibration, standards were prepared by using a different amount of pure γ-oryzanol (5, 10, 25 and 40 mg/L) in methanol and calibrated curve and correlation

constant were determined for total γ-oryzanol. The obtained calibration curve and correlation coefficient (R^2 = 0.9948) are shown in Annex 3. Whereas HPLC chromatograph for 40 mg/L γ-oryzanol is shown in Annex 4.

Mobile phase composition was a mixture of methanol and acetonitrile (75:25) with a flow rate of 1 mL/min. While injection volume was 50 µm. A C18 inertsil ODS column (4.6 mm, 250 mm, 5 µm) with DAD wavelength (325 nm) was used for chromatographic separations of γ-oryzanol. Results were determined for rice only and expressed as mg γ-oryzanol/kg of dry matter, after duplicate analyses. Whereas, percent composition of γ-oryzanol (ferulates) was calculated by dividing the individual area by the total area.

3.9. Water Holding Capacity

Water holding capacity (WHC) was calculated according to Sahan et al. [110] with minor modifications. Briefly, ~10 g yogurt sample (Yw) was centrifuged for 20 min at 5000 rpm and 4°C. The supernatant (whey) was removed and weight of remained pellet (Pw, g) was measured.

The WHC (expressed in percentage) was determined as;

$$WHC\ (\%) = \frac{Pw}{Yw} \times 100$$

3.10. Textural Analyses

Texture analysis of yogurt was determined according to Najgebauer-Lejko et al. [111]. Texture measurements were carried out by Texturometer model TA-PLUS (Lloyd, UK), where back extrusion test (BET) was performed by using a probe of 0.5 cm diameter. Different parameters such as hardness, gumminess, cohesiveness, and springiness, which provide information about textural properties of yogurt samples were obtained from Nexygen 2.0 (Lloyd, England) by using force versus time curve graphs (TPA curve).

3.11. Measurement of Antioxidant Capacity

The total antioxidant activity of samples (rice and yogurt) were analyzed by “QUENCHER” method as described by Serpen et al. [112]. About 10 mg GBR or 60 mg fresh yogurt sample was mixed with 10 mL working solvent of $ABTS^{\cdot+}$ radical and shaken for 27 min at 350 rpm, centrifuged (Sigma3-18K, Germany) for 2 min at

8000 rpm. Finally, absorbance of the supernatant was measured by spectrophotometer at the wavelength of 734 nm.

First stock solution of $ABTS^{\cdot+}$ radical was prepared in a final concentration of 7 mmol/L ABTS and 2.45 mmol/L $K_2S_2O_8$ and allowed to stand in dark place for about 15 hours at room temperature. Then $ABTS^{\cdot+}$ working radical solvent was prepared by diluting the stock solution with the mixture of ethanol and deionized water (in the ratio of 1:1) till absorbance of 0.70-0.80 at 734 nm. Standard Trolox solutions were made in concentration range of 0, 100, 200, 300, 400, 500 and 600 µg/mL Trolox in methanol. A standard calibration curve was determined by mixing 0.1 mL standard solution of each concentration with 10 mL $ABTS^{\cdot+}$ working radical solvent and their absorbance was measured at 734 nm. The obtained calibration curve and correlation coefficient ($R^2 = 0.9976$) are shown in Annex 5.

Individual duplicate samples were analyzed and total antioxidant activity was determined by comparing standard curve. All results were represented as Trolox equivalent antioxidant capacity present in 1 kg dry matter (mmol TEAC/kg dry matter).

3.12. Sensory Evaluation

Sensory evaluation was determined according to Peryam and Girardot [113] with minor modifications. Briefly, 12 trained panelists consist of faculty members and postgraduate students of the Food Engineering Department, Hacettepe University Ankara, were requested to evaluate the appearance, texture/flavor, color and overall acceptability of the prepared yogurt samples. The assessments were represented on the 9-point hedonic scale ranging from 9 (“like extremely”) to 1 (“dislike extremely”). The used sensory evaluation scorecard is attached in Annex 6.

3.13. Statistical Analysis

All duplicate results were expressed in the form of mean ± standard deviation after analyzing with SPSS 23.0 software (SPSS, Inc., Chicago, Ill., USA).) Also, one-way analysis of variance (ANOVA) has been used in order to study the effect of germination and fermentation on the GABA content, total γ-oryzanol, WHC, texture and total antioxidant capacity. The differences between means were conducted by using DUNCAN’s multiple range test and results with $p < 0.05$ were considered statistically significant.

4. RESULTS AND DISCUSSION

4.1. Brown Rice

Brown rice (BR) have more nutritional benefits and low glycemic index as compared to white rice (WR) [114]. Loss of these beneficial effects in WR occurs with the removal of bran layers during the polishing process [115]. However, consumption of BR is lower than WR, mainly due to the poor eating texture. But in recent years, the popularity of brown rice is increasing because of their higher antioxidant and fiber contents [116]. Also, germination is recognized as an efficient method to improve the texture and palatability of cooked brown rice [27].

In this study, BR (*Japonica* varieties of *Oryza sativa L.*) were purchased from local market. And instead of using sodium hypochlorite (NaOCl) for the reduction of microbial load, BR was surface cleaned by rinsing with enough amount of distilled water. Because previous studies reported that NaOCl as a strong oxidant has caused adverse effects on γ-oryzanol contents and total antioxidant capacity of brown rice [117, 118]. In another scale-up study, hot water (90°C) treatment for 90 sec was found to be effective for the elimination of pathogens, but it significantly reduced germination yield [119]. This could be explained by partial inactivation of the hydrolytic enzymes responsible for germination. Many bio-functional components and phytochemicals such as GABA, γ-oryzanol, vitamins, minerals, tocopherols, flavonoids, phenolic acids and proanthocyanidins in bran layer of BR can be increased through germination process [120]. Therefore, distilled water was used at room temperature without any disinfectant for the efficient germination of brown rice.

4.2. Germinated Brown Rice

After proper cleaning, brown rice was soaked in distilled water (1:3, w/v) and germinated for 96 hours. In order to prevent or reduce microbial load, replacement of equal quantity of distilled water was repeated after regular intervals. Also, samples were taken after every 12 hours and oven dried at 30°C.

An elongation in sprouts was observed by increasing germination time, as shown in Figure 4.1. At the end of germination, sprouts length was increased up to 24 mm. Some studies reported a linear relation between sprouts length and GABA

production [34, 121]. However, Lu et al. [42] found a negative relation between GABA synthesis and length of sprouts.

Figure 4.1. The sprouting of GBR at different hours of germination.

Composition analyses of brown rice before and after germination are presented in Table 4.1**.** The results indicated that total solids of BR increased during germination. Normally BR have hygroscopic nature due to expendability of starch cells with water absorption and moisture contents less than 15% [122], which is similar to our results. In a germination study, Xu et al. [31] determined breakdown of starch molecules by enzymes, which was already explained in section 2.3.

In GBR samples, total solids contents increased with degradation of starch molecules, as water absorption was decreased. Also further increase in total solids contents were observed with prolonged germination. While protein and fat contents increased accordingly to the total solids.

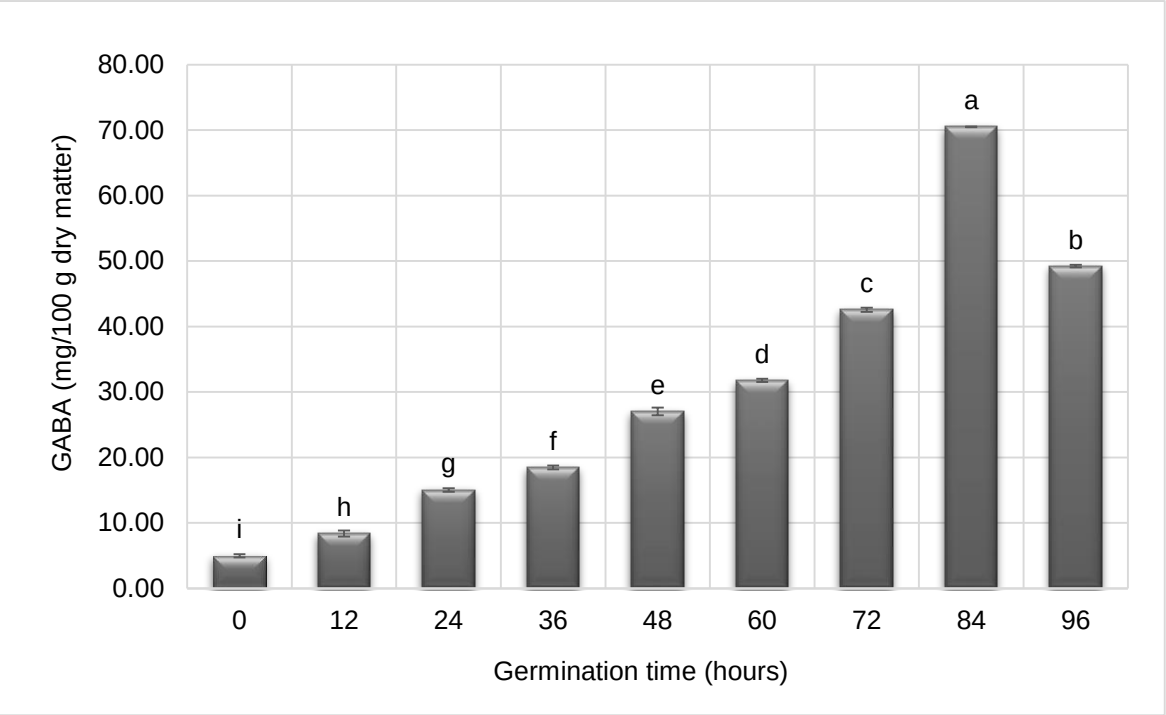
Table 4.1. Composition (%) of brown rice before and after germination.

Germination Time (hours)	Total solids*	Protein*	Fat	Ash*
0	87.67 ± 0.17	9.38 ± 0.13	2.19	1.26 ± 0.03
12	94.42 ± 0.07	10.73 ± 0.20	2.38	1.34 ± 0.04
24	94.32 ± 0.20	10.73 ± 0.11	2.40	1.25 ± 0.05
36	94.54 ± 0.30	11.08 ± 0.18	2.57	1.21 ± 0.02
48	94.80 ± 0.18	10.73 ± 0.48	2.51	1.09 ± 0.06
60	94.63 ± 0.47	10.46 ± 0.12	2.51	1.02 ± 0.04
72	95.36 ± 0.14	11.04 ± 0.41	2.63	1.04 ± 0.07
84	95.27 ± 0.11	11.37 ± 0.14	2.68	0.96 ± 0.01
96	94.69 ± 0.42	11.19 ± 0.12	2.79	1.01 ± 0.02

***** Values are the mean ± standard deviation of duplicate experiments.

4.2.1. γ-aminobutyric Acid Content in GBR

In non-germinated brown rice (NGBR), GABA has been found to be 4.96 mg/100 g dry matter. Brown rice generally contain GABA in the range of 1-10 mg/100 g dry matter, depending upon rice cultivars, as described by related literature [123, 124]. GABA content of brown rice significantly increased with germination ($p < 0.05$), as shown in Figure 4.2. Where highest GABA content was observed in GBR after 84 hours (70.50 ± 0.04 mg/100 g dry matter), which is about 14 times higher than NGBR. Similar results were presented by Ohtsubo et al. [125], who reported an increase in GABA as 11.5 folds to NGBR, after 72 hours of germination.



a-h, Different lowercase letters show statistical difference among GBR types ($p < 0.05$)
Error bars are used for the standard deviation (SD ± 2) of duplicate samples.

Figure 4.2. Effect of germination period on GABA contents.

GABA is produced by α-decarboxylation of L-glutamic acid or glutamate, in the presence of glutamate decarboxylase (GAD) enzyme and its cofactor pyridoxal 5-phosphate (P5P) [126]. During germination, water uptake improved enzymatic and proteolytic activities. As a result, starch decomposed into smaller oligosaccharide, disaccharide and monosaccharide. Whereas, protein degrades into polypeptides, peptides and amino acids. Therefore, maximum degradation of proteins with free amino acids (e.g. glutamic acid) and low pH may be the reasons for increased rate of GABA production at 84 hours. And an inverse relation was observed between GABA content and glutamic acid during germination, where low pH has a positive

influence on the decarboxylation of glutamic acid [127]. The decrease in GABA content at 96 hours of germination may be attributed to the reduction of glutamic acid or GAD enzyme concentration. In another study, Liu et al. [121] observed the effects of different pH solvents on the germination process. And as compared to tap water, 39.76% rise in GABA content was reported by using strong acidic electrolyzed water. Also, it has been reported that pH 5.0-6.0 was ideal for GABA production at 40°C after soaking for 4 hours [27].

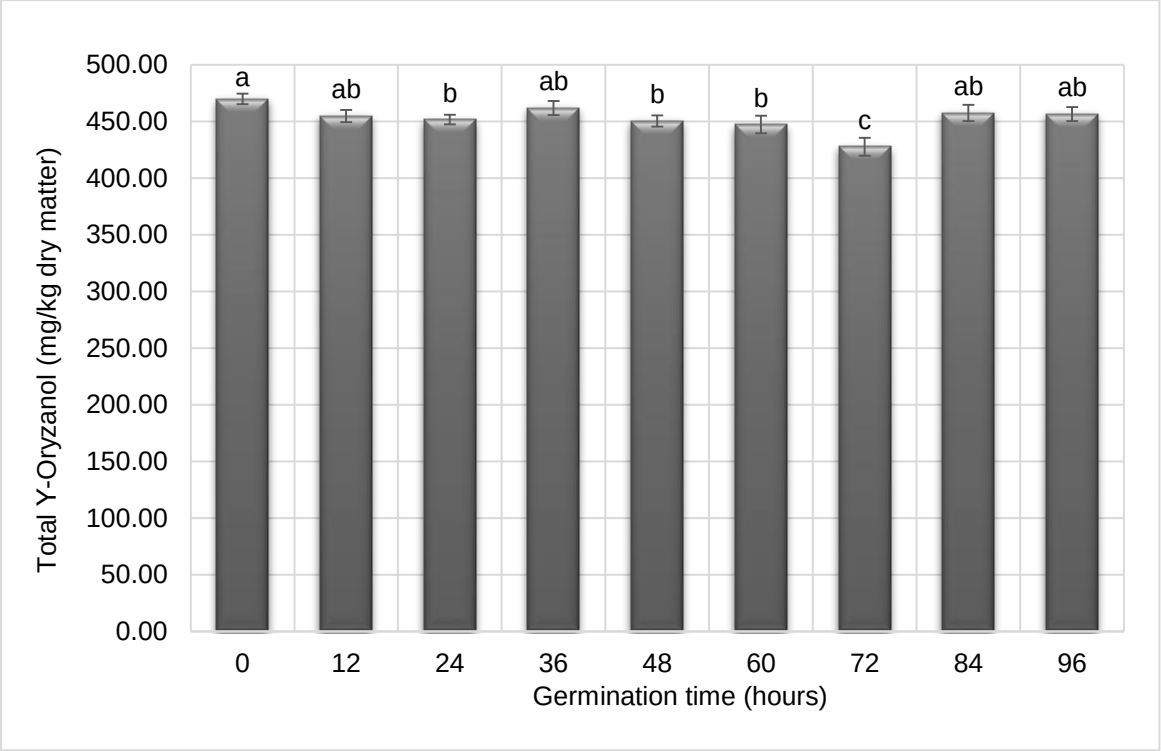
In our study, reduction in pH was observed (generally from ca. 6.0 to 4.0) in replaced water after every 4 hours of germination. This pH decrease might be explained by the production of GABA. Water replacement during germination maintained the pH values above 4.0. Otherwise, GABA production could be affected as GAD enzyme has maximum activity at pH 6.0 [128].

4.2.2. Total γ-oryzanol Contents in GBR

γ-oryzanol is a combination of trans-ferulic acid esterified with sterols or triterpene alcohols. The total amount of γ-oryzanol is expressed as the sum of four basic ferulate components (cycloartenol ferulate, 24-methylene cycloartenol ferulate, β-sitosteryl ferulate and campesteryl ferulate).

Overall γ-oryzanol contents did not change significantly with germination in general, as shown in Figure 4.3**.** Oryzanol contents have decline statistically from the beginning of germination to 24 hours and this reduction was ca. 4%. After 72 hours of germination, the reduction of total γ-oryzanol (ca. 9%) was found to be significant ($p < 0.05$).

During germination, high activity of feruloyl esterase may be the reason of oryzanol reduction, which causes the release of the ferulic acid component by hydrolyzing the esters of phenolic acids [129]. Some studies reported no difference in total oryzanol after germination [57, 125]. While other investigations revealed that γ-oryzanol contents increased after germination [126, 130]. On the other hand, the effect of germination on γ-oryzanol was studied by Kiing et al. [131], who germinated eight different rice cultivars for 24 hours, and slight oryzanol increase was observed in only three of them while it reduced in rest of the cultivars. These differences in γ-oryzanol contents may arise due to the changes in germination conditions and varieties of brown rice.



a-c, Different lowercase letters show statistical difference among GBR types ($p < 0.05$)
Error bars are used for the standard deviation (SD ± 2) of duplicate samples.

Figure 4.3. Effect of germination time on total γ-oryzanol.

The germination process has also influenced percent composition of total γ-oryzanol, as described in Table 4.2.

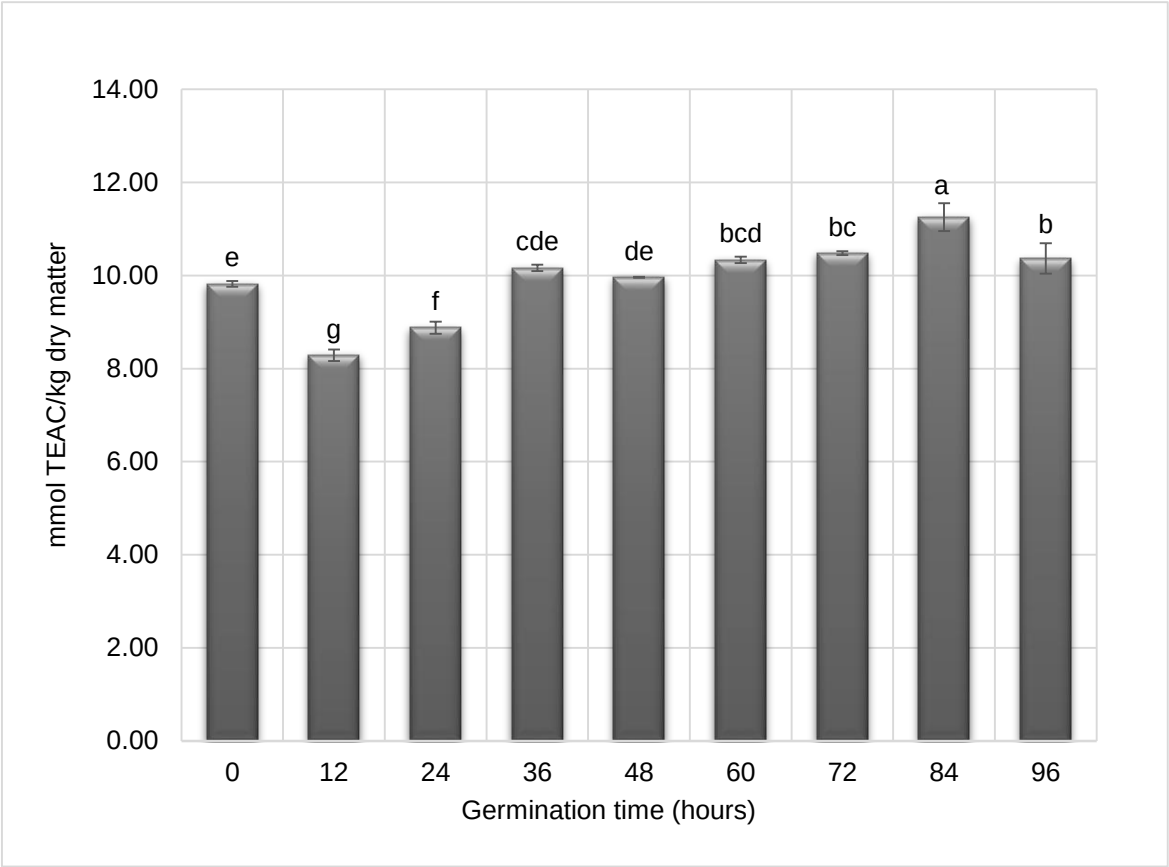
Table 4.2. The effect of germination on (%) components of γ-oryzanol.

Germination Time (hours)	Cycloartenol ferulate	24-methylene cycloartenol ferulate	β-sitosteryl ferulate	Campesteryl ferulate
0	35.4	32.9	18.6	13.1
12	35.4	33.2	18.5	12.9
24	34.9	33.9	18.3	12.9
36	35.5	33.9	18.2	12.3
48	35	33.9	18.4	12.7
60	35.3	33.5	18.4	12.9
72	35.3	34	18.3	12.5
84	35.6	33.8	18.1	12.4
96	35.5	34	18.1	12.4

The amounts of cycloartenol ferulate and 24-methylene cycloartenol ferulate were increased after 96 hours of germination, while campesteryl ferulate and β-sitosteryl ferulate reduced as compared to NGBR. Literature is very limited for the explanation of germination effects on the composition of total γ-oryzanol [118].

4.2.3. Effect of Germination on Total Antioxidant Capacity

In this study, total antioxidant capacity was measured by “QUENCHER” method and calculated as Trolox equivalent antioxidant capacity (mmol TEAC/kg dry matter). The changes in the antioxidant capacity of GBR is shown in Figure 4.4**.** The total antioxidant capacity of brown rice decreased during the first 12 hours of germination, then increased up to 84 hours with little deviations ($p < 0.05$).



a-e, Different lowercase letters show statistical difference among GBR types ($p < 0.05$).
Error bars are used for the standard deviation (SD ± 2) of duplicate samples.

Figure 4.4. The changes in total antioxidant capacities during germination.

These variations in total antioxidant capacity can be attributed to redox biology of the plants. During germination, hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS) such as superoxide anions are continuously increased with the imbibition of water, caused oxidative stress, and result in reduced antioxidant capacity.

Then rise in antioxidant activity was observed after 24 hours of germination. Antioxidant enzymes are being produced in water absorbed cells by prolongation of germination period, in order to maintain the balance of redox homeostasis. These antioxidant enzymes (peroxidase, superoxide dismutase and catalase etc.) quench the produced ROS and oxidized proteins. They also protect the cells from oxidative damages.

The change in color of grains occurred by over-accumulation of ROS. Black spots in rice grains were appeared due to the superoxide anion and bleaching was caused by H_2O_2. As shown in Figure 4.5. (A), He et al. [132] showed the color difference by ROS and similar observation were demonstrated by our findings, as in Figure 4.5.(B).

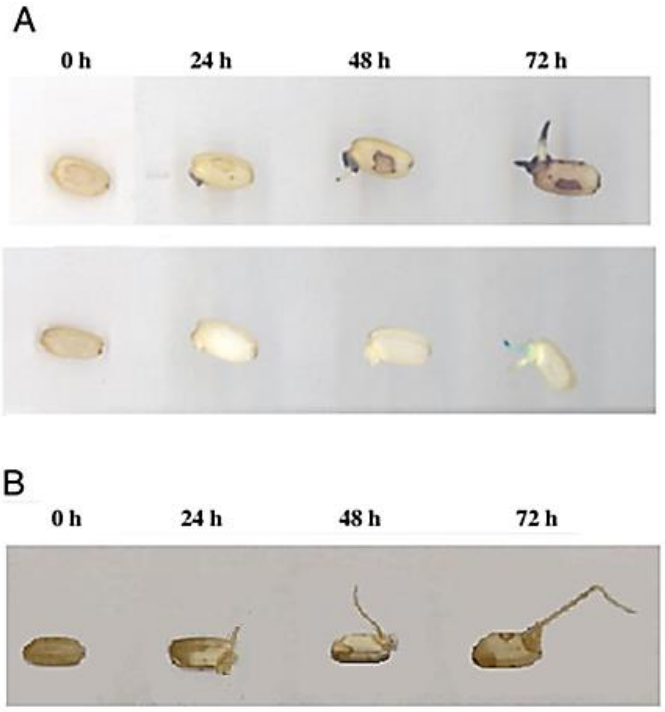


Figure 4.5. The accumulation of ROS during germination of brown rice; A: findings of He et al. [132] and B: results of this thesis.

On the other hand, the total antioxidant capacity of brown rice is also significantly affected by the presence of oryzanol and phenolic acids compounds [129, 133]. It is well known that oryzanol is a very strong antioxidant compound found in rice bran oil. Brown rice is a very important source of γ-oryzanol, which was preserved during germination (described in Section 4.2.2). Since disinfectant (hypochlorite) as a cleaning agent was not used before germination, as it has adverse effect on total γ-oryzanol contents.

4.3. Yogurt

4.3.1. Physicochemical Properties of Milk

Pasteurized milk prepared for yogurt production was purchased from A.O.Ç and the results of physicochemical analyses of milk were given in Table 4.3.

Table 4.3. The physicochemical properties of milk used for yogurt preparation.

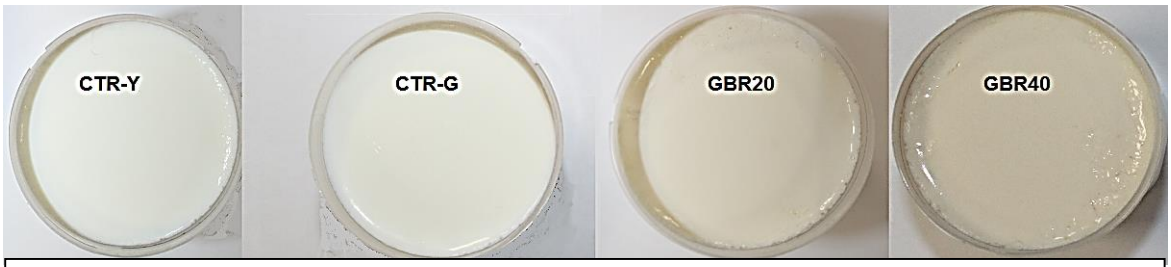
pH / Temperature (°C)	6.76 / 11
Titratable acidity* (%Lactic acid)	0.15 ± 0.006
Total Solids* (%)	12.23 ± 0.010
Fat* (%)	3.05 ± 0.071
Protein* (%)	3.58 ± 0.079
Ash* (%)	0.67 ± 0.228

*Values are the mean ± standard deviation of duplicate experiments.

4.3.2. Yogurt Production

In this study, set type yogurt samples were produced with GABA fortification. Two types of control yogurt samples were prepared; first control was conventional yogurt (CTR-Y) and second control was GABA standard containing yogurt (CTR-G). As no GABA was detected in control CTR-Y yogurt, so it cannot be used for comparison of GABA fortified yogurt samples. But CTR-Y yogurt was used for comparison of antioxidant capacity, textural and sensory evaluations. While other control (CTR-G) was only used for the comparison of GABA contents in yogurt samples containing GBR crude extracts.

After fermentation, images of all types of yogurt samples (with and without GABA fortification) are presented in Figure 4.6**.** As it can be seen that color and overall appearance are different among yogurt samples. Development of brown color in yogurt was observed with the addition of GBR crude extracts. As water extracts of GBR flour were sterilized for safety reasons and freeze-dried before its addition to milk samples. This high temperature sterilization also caused the development of Maillard browning in obtained extracts.



CTR-Y (control yogurt), CTR-G (yogurt with GABA standard), GBR20 (yogurt with crude extract of 20 g GBR) and GBR40 (yogurt with crude extract of 40 g GBR).

Figure 4.6. Images of different types of yogurt samples.

In pre-experiments, different concentrations (1-5%) of GBR flour were added to the milk and used for the production of set type yogurt. GABA was not detectable even at a maximum concentration of 5%. Although very fine particles of GBR flour were used and homogenized (at least for 5 min at 15000 rpm), but slow precipitation was observed during fermentation (even with the addition of 1% flour). Yogurt texture was extremely affected by the increase of GBR flour. For improvement of yogurt texture, crude extracts were obtained from the GBR flour and used for GABA fortification. After germination of 84 hours, GBR flours in the quantity of 20 and 40 g were found suitable for the extraction of enough amount of GABA and yogurt production with improved texture.

4.3.3. Effect of Storage Period on Total solids and pH of Yogurt Samples

In all yogurt samples, the effect of storage period (21 days) on the changes in total solids are shown in Table 4.4. An increase in total solids were observed with both GABA addition and storage time. Among yogurt types, this increase can be elaborated with rise in dry matter by addition of GABA standard or extracts. Whereas the evaporation phenomenon can explain the increase in total solids with storage period.

Table 4.4. Effect of storage conditions on total solids (TS*) of yogurt samples.

Days / Types	1	7	14	21
CTR-Y	12.28 ± 0.01	12.33 ± 0.001	12.42 ± 0.03	13.09 ± 0.22
CTR-G	12.76 ± 0.06	12.86 ± 0.03	12.88 ± 0.03	13.12 ± 0.02
GBR20	12.38 ± 0.02	12.50 ± 0.03	12.53 ± 0.01	12.58 ± 0.09
GBR40	12.46 ± 0.05	12.64 ± 0.02	12.68 ± 0.09	12.69 ± 0.03

*Values are the mean ± standard deviation of duplicate experiments.

Also, pH changes were monitored in yogurt samples during the storage period, as shown in Table 4.5. When the pH reached to 4.60, the fermentation was terminated by rapid cooling to 4°C. After 1 day of storage, pH of control yogurt (CTR-Y) dropped to 4.56. Also, a slow reduction in pH was perceived for all yogurt samples during storage of 21 days. It shows the continuous production of lactic acid by yogurt culture even at 4°C.

Table 4.5. Effect of storage conditions on pH of yogurt samples.

Days / Types	1	7	14	21
CTR-Y	4.56	4.41	4.26	4.19
CTR-G	4.72	4.43	4.31	4.25
GBR20	4.50	4.28	4.19	4.13
GBR40	4.44	4.25	4.15	4.10

A rise in pH of yogurt was observed after the addition of GABA standard in CTR-G yogurt. Similar results were reported by Liu et al. [101], who noticed higher pH value (4.90) for yogurt samples fortified with 1% GABA.

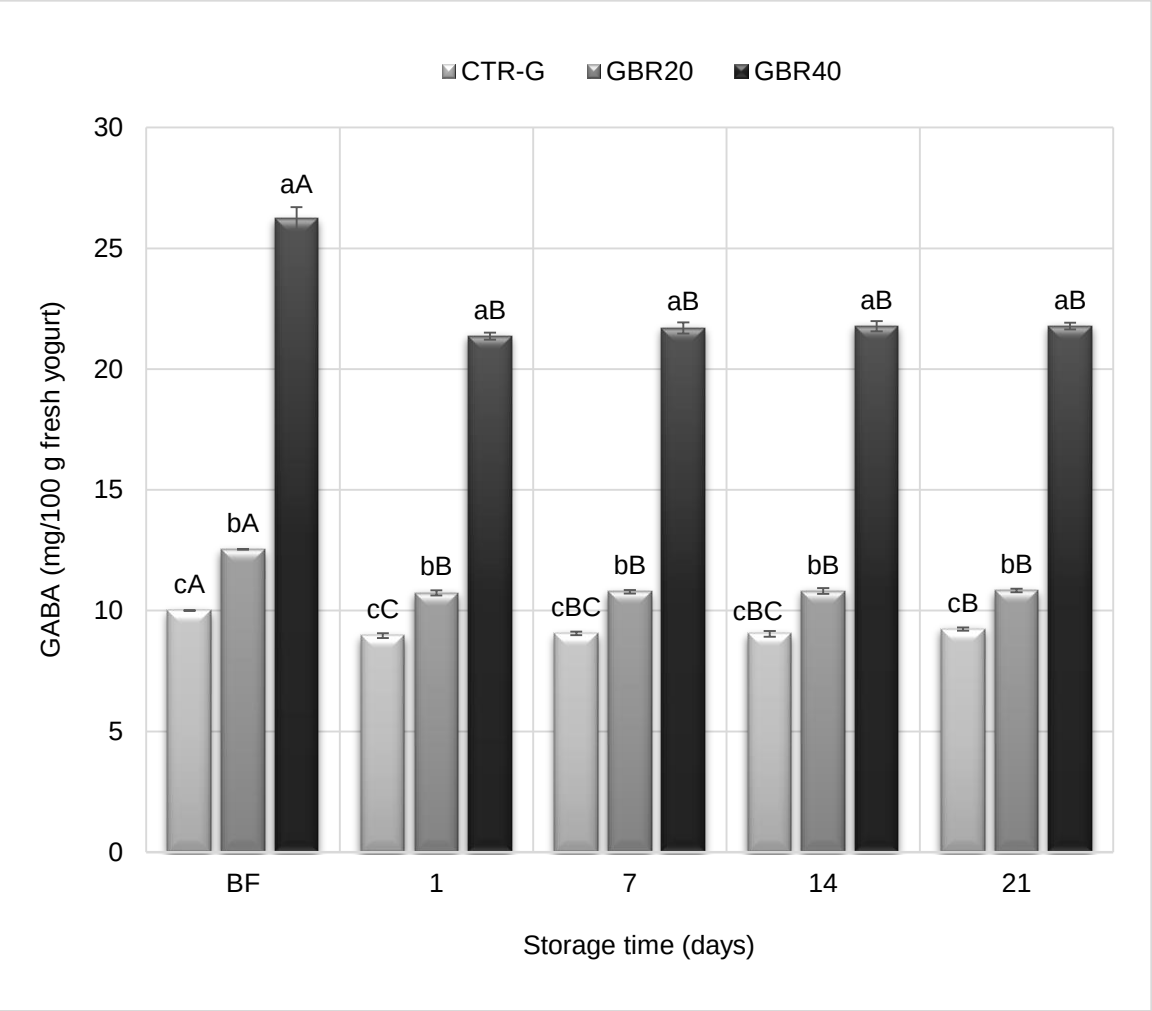
On the other hand, pH values reduced for yogurt samples containing crude GABA extracts. This decrease could be explained by the presence of reducing sugars in GBR crude extracts, which may be responsible for the increased acid development during fermentation.

4.3.4. GABA Fortified Yogurt

GABA content was not detected in the production of control yogurt (CTR-Y), therefore it was not used for the comparison. Other control yogurt (CTR-G) was prepared with standard GABA for the evaluation of GABA fortified yogurt samples. Before fermentation (BF), 10 mg of GABA standard was added into 100 g milk for CTR-G yogurt and 12.54 and 26.24 mg of GABA in the form of crude extracts (pre-calculated by HPLC) were added into 100 g milk samples for GBR20 and GBR40 yogurt samples, respectively.

After fermentation GABA reduction of about 10.36, 14.39 and 18.57% was observed in CTR-G, GBR20 and GBR40 yogurt samples, respectively ($p < 0.05$), as shown in Figure 4.7. Similar results were reported by Anawachkul and Jiamyangyuen [100], who manufactured GABA enriched yogurt, and about 24% loss was observed in

GABA quantity after fermentation. On the other hand, Liu et al. [101] have described that yogurt processing conditions (fermentation) have no significant effects on the GABA content. This statement is conflicting to other studies of the same group, where they reported that many factors like heating, homogenization and milk fractions (casein, whey, and lactose) have a negative influence on the GABA content. They also observed the formation of GABA dimers due to the self-reaction process, which may be another reason for GABA loss [4, 134].



a-c, Different lowercase letters show statistical difference among yogurt types ($p < 0.05$)
A-B, Different uppercase letters show statistical difference among storage days ($p < 0.05$)
Error bars are used for the standard deviation (SD ± 2) of duplicate samples.

Figure 4.7. Changes in GABA contents of yogurt during storage.

Most GABA manufacturing microorganisms are LAB but their GABA producing abilities are only limited to strains that contain active GAD enzyme [36]. Turkish standard only approved *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* as the culture bacteria for the production

of yogurt. Both of these bacteria work in symbiotic manners, where the growth of one bacterium is influenced by the substances produced by other bacterium. Firstly, *St. thermophilus* consume simple sugars and free amino acids for the fulfillment of their growth requirements and create acidic (up to pH 5) and anaerobic conditions by producing formic acid, lactic acid, and CO_2. Then, development of these conditions stimulates the growth of *Lb. bulgaricus*, which have more proteolytic activities and abilities to produce various amino acids such as glutamic acid, valine, tryptophan, histidine, leucine, and isoleucine. Therefore, the growth of *Streptococcus thermophilus* again increased by consuming these amino acids. In a study, Tamime and Robinson [135] reported that diminishing these amino acids in isolated media, results in growth reduction of *St. thermophiles* by about 50%. Because of the structural similarity with glutamic acid, added GABA in milk may be thought to be consumed by *St. thermophilus* during fermentation.

There is a limited literature for the explanation of the GABA reduction by bacterial fermentation. During ethanol fermentation in sake brewing, Ando and Nakamura [136] explained the utilization of GABA by yeast (*Saccharomyces cerevisiae*) as a source of nitrogen. Also, 28.2% loss in GABA content was observed during fermentation of cocoa beans [137]. On the other hand, Watanabe et al. [138] studied the effects of co-culturing yogurt bacteria on GABA production and reported that *Streptococcus thermophilus* has the ability to produce GABA in 1% monosodium glutamate (MSG) medium at 3.50 pH. Whereas, in our study, MSG was not used in yogurt production and also fermentation was rapidly stopped at 4.60 pH according to the procedure of yogurt production.

On the other hand, storage period of 21 days has no significant effect on the GABA quantity. These results revealed that the GABA as a valuable bioactive compound can be preserved until the consumption of yogurt (Figure 4.7). This is also an important attribute for the manufacturing of GABA fortified functional yogurt on commercial basis.

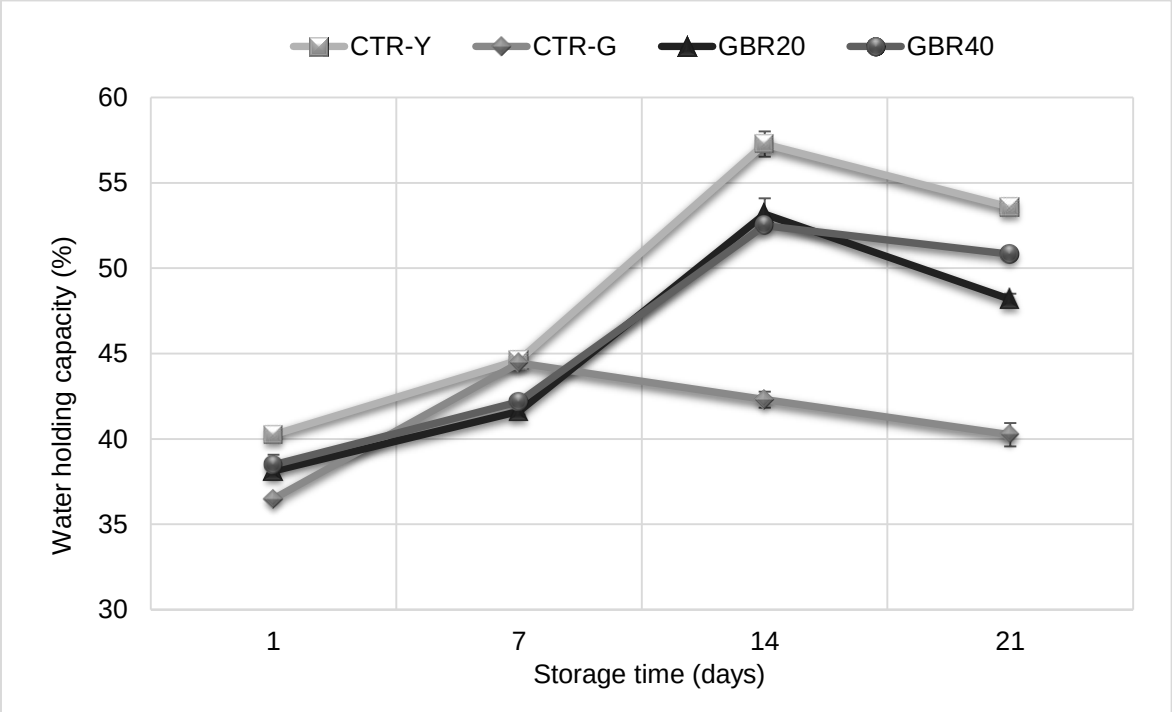
In a study, Inoue et al. [44] testified that daily intake of 10 mg GABA containing fermented milk for the period of 3 months was effective for the individuals with high blood pressure. In our findings, consumption of only one portion of GABA fortified yogurt with crude extract of 20 g GBR can deliver more than 10 mg of GABA in 100 g of fresh yogurt. For further studies, the bioavailability of GABA in human

gastrointestinal tract has to be investigated with the consumption of commercial GABA fortified yogurt.

4.3.5. Water Holding Capacity

Water holding capacity (WHC) is an important parameter for consumer's acceptability of yogurt during the storage period. It also reflects the degree of fermentation and stability of protein gel network (curd). WHC is often defined by the percentage of concentrated yogurt (w/w) obtained by static or dynamic (centrifugation) drainage.

WHC of all GABA fortified yogurt samples is significantly lower than control CTR-Y yogurt ($p < 0.05$), as shown in Figure 4.8. This reduction may be correlated with high pH of yogurt with the addition of GABA, which negatively affect the protein gel formation. According to Turkish standard, fermentation was stopped in control CTR-Y yogurt at the pH of 4.60 after about 4 hours. Therefore, for comparison, fermentation for all yogurt samples was terminated according to control CTR-Y yogurt. In yogurt production, casein and whey (β-lactoglobulin) are major proteins, which have isoelectric points of about 4.6 and 5.3, respectively [74]. At the isoelectric point of 4.60 pH, casein protein aggregates into a gel type structure, which can entrap water inside the three-dimensional network.



Error bars are used for the standard deviation (SD ± 2) of duplicate samples.

Figure 4.8. Effect of GABA fortification on WHC of yogurt samples with storage.

Addition of GABA standard caused an increase in pH of the CTR-G yogurt after fermentation, and consequently reduced the curd firmness and WHC. However, GABA containing GBR crude extracts consist of many reducing sugars from degradation of starch by germination, which caused an increase in the acidity (pH lower than that of control CTR-Y) of enriched yogurt samples. For this reason, a similar trend was observed in WHC of control CTR-Y and GABA fortified yogurt samples (GBR20 and GBR40) during the storage period.

After storage of 7 days, no significant difference in WHC of control CTR-Y and CTY-G was detected because of rapid reduction in pH of CTR-G yogurt. This may be explained by increased lactic acid production by the LAB at high pH (4.72), which is more than the pH (4.56) of control CTR-Y at first day of storage (Table 4.5). In a study, reduction in WHC of yogurt samples was also described by Liu et al. [101] with increased addition of GABA.

Some studies observed an increase in WHC of yogurt with the addition of rice and rice bran flour. This improvement can be the result of high water binding capacity of fiber contents in rice flour [139, 140].

On the other hand, WHC was increased in all yogurt samples with storage period of 14 days and finally decreased at 21 days. Here an increase in acidity improved the WHC till 14 days then little reduction was detected, which may be explained by weakness in protein gel because of over-acidification and increased casein solubility at pH (~4.20), which is lower than the isoelectric point of casein. Similar results for the water holding capacity of Turkish type low-fat yogurt were also reported by Emirdağı [96].

4.3.6. Textural Analyses for Yogurt Samples

Texture represents the rheological, microstructural and sensorial properties of food. Textural characteristics of yogurt (hardness, gumminess, cohesiveness, and springiness) were affected by various parameters such as the composition of milk, the intensity of heat treatment, quantity and type of starter culture, fermentation temperature and storage conditions [81]. Hardness is a peak force required for product deformation during the first cycle of compression. And, the energy required to break a semisolid food-stuff into a ready-to-swallow state is defined as gumminess [141]. Whereas, cohesiveness is the rate of the applied force-areas

between the second cycle of compression and the first cycle of compression. The length for which the food sample regain its height throughout the time interval that passes in-between the completion of first cycle of compression and the biginning of the second cycle of compression is defined as springiness [142].

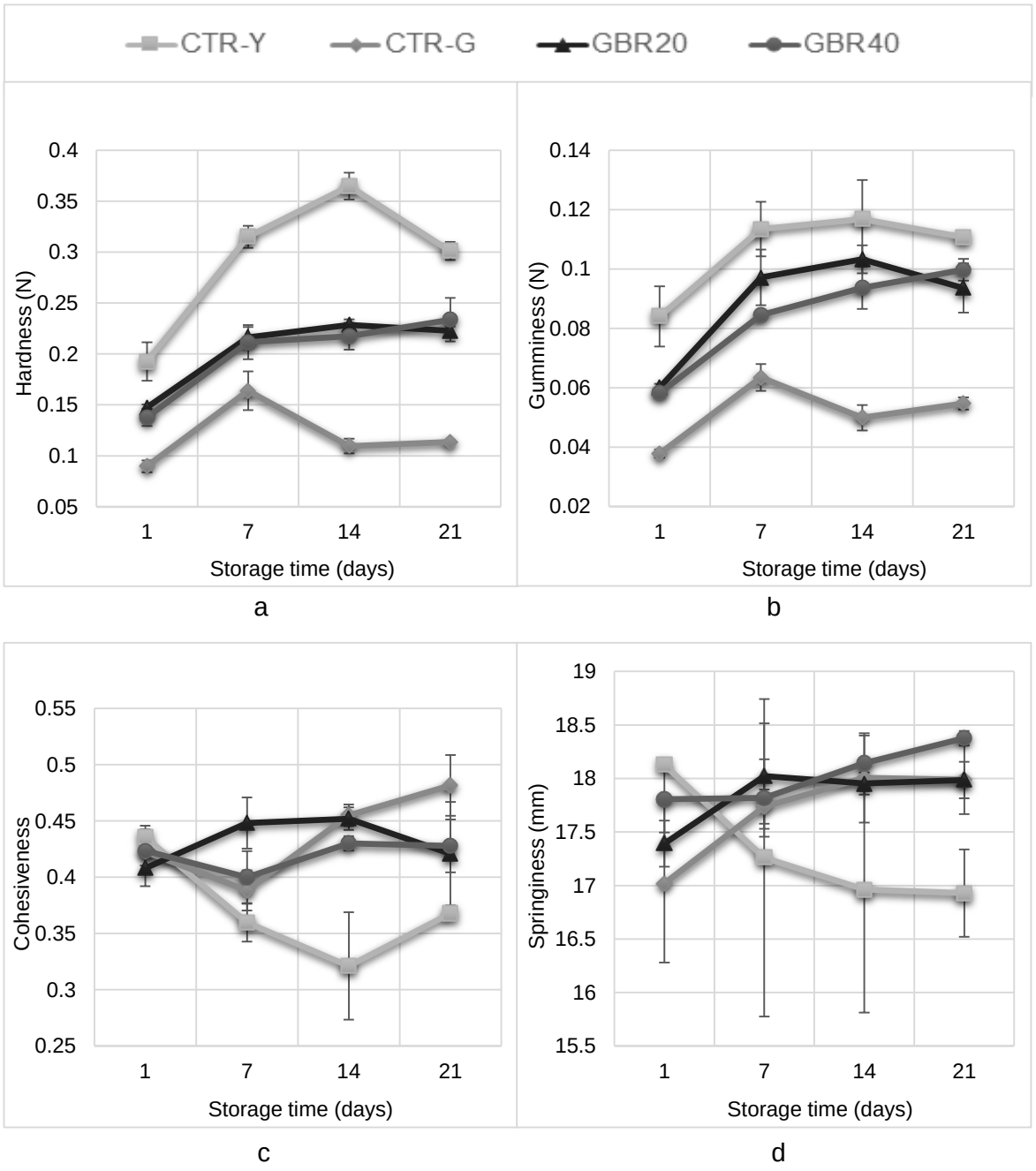
The textural profiles of yogurt samples were determined during the storage period of 21 days. For all types of yogurt samples, hardness and gumminess gradually rise until 14 days of storage then slightly declined, as shown in Figure 4.9 (a & b). All GABA fortified yogurt samples have lower values of hardness and gumminess than the control CTR-Y. A similar phenomenon was observed for the WHC of yogurt samples, as reported in section 4.3.5.

The results of textural analyses have indicated that pH has a prominent role in the textural parameters, as it was expected. Initially, hardness and gumminess increased while pH reduced to around 4.30 and a further drop in pH (4.20) has decreasing effects on them (Table 4.5). The factors like total solids and polysaccharide directly influenced the texture of yogurt by increasing water holding capacity. There is a linear relation between WHC and improvement of hardness and gumminess. Hardness and gumminess were also affected by storage period.

As compared to control CTR-Y, non-significant increase was observed for cohesiveness and springiness of all GABA fortified yogurt samples during storage ($p > 0.05$), as shown in Figure 4.9 (c & d). This indicates that a very little curd deformation occurred in GABA fortified yogurt samples.

For control CTR-Y yogurt, cohesiveness and springiness were reduced non significantly. Similar results were determined in yogurt by El-Gammal et al. [143] and they justified this reduction by high protein proteolysis. They also concluded an inverse relation of hardness and gumminess to cohesiveness and springiness during storage, as this has been justified by our study too.

GABA enrichment has a positive impact on protein structure (less deformation in curd) and consequently, it improved yogurt texture a little bit, as compared to control CTR-Y yogurt during the storage period.



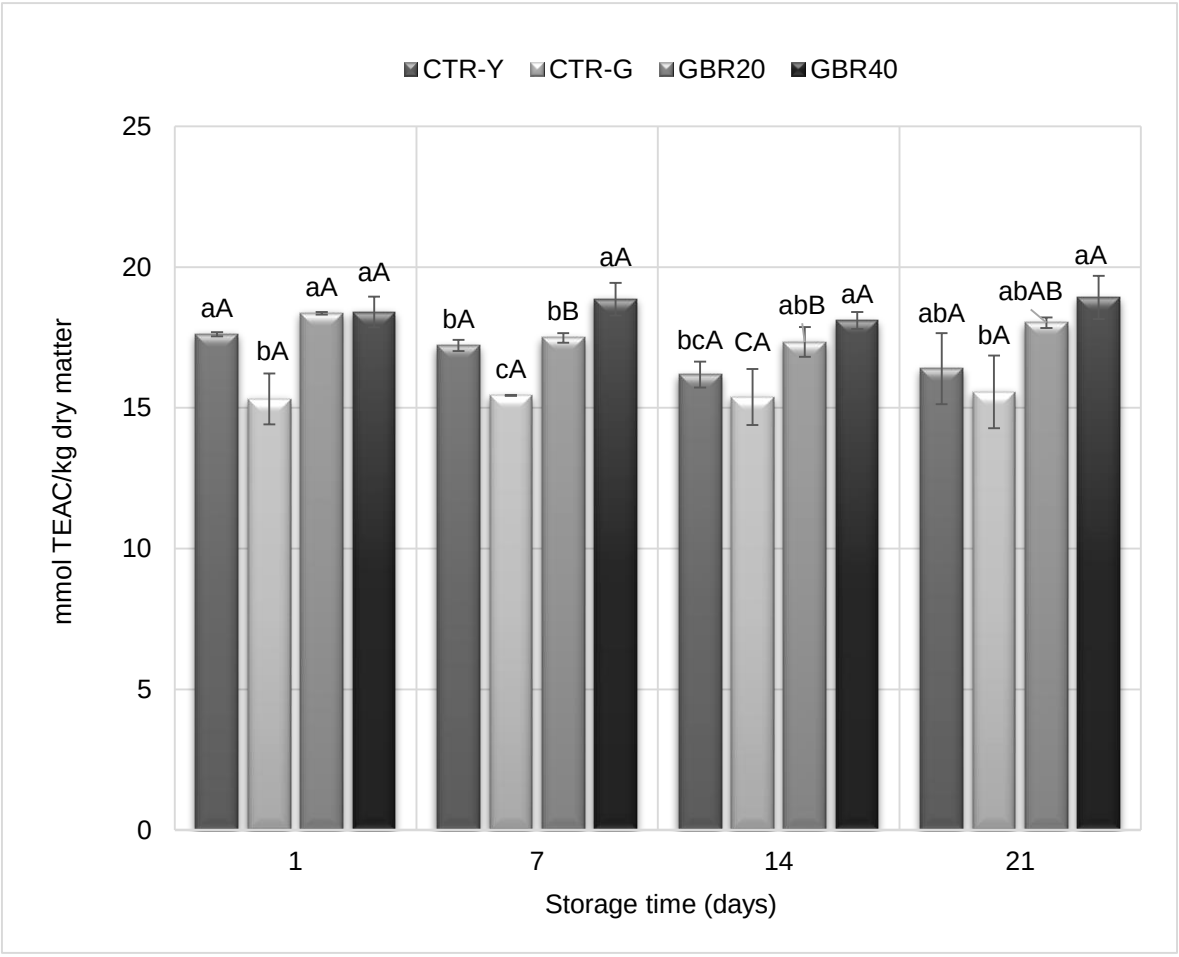
Error bars are used for the standard deviation (SD ± 2) of duplicate samples.

Figure 4.9. Textural analysis of GABA enriched yogurt

4.3.7. Total Antioxidant Capacity in Yogurt

Fermented dairy products have been identified as the dietary source of natural antioxidants due to the presence of bioactive peptides having antioxidant properties. Most of these peptides derived from milk proteins as a result of LAB fermentation. During the production of yogurt, pH of milk was reduced from 6.76 to 4.60 which caused the breakdown of casein protein into many bioactive peptides. Therefore, the antioxidant capacity of yogurt changes with storage period [144].

During storage, total antioxidant capacities in all yogurt samples were changed non-significantly ($p > 0.05$), as shown in Figure 4.10. Initially, total antioxidant capacities decreased insignificantly until the end of 14 days then slightly increased at 21 days of storage. Similar results were reported by another study, where aqueous extracts were obtained from plain and skim type yogurt samples for the measurement of antioxidant capacities, which reduced in first 5 weeks of storage then little increase was perceived during next two weeks [145]. However, Muniandy et al. [146] observed no effect of fermentation on the antioxidant capacity of plain yogurt. But, in some studies, it was reported that antioxidant capacity in water-soluble peptide extracts of fresh yogurt increased gradually during storage [144, 147].



a-c, Different lowercase letters show statistical difference among yogurt types ($p > 0.05$)
A-C, Different uppercase letters show statistical difference among storage days ($p > 0.05$)
Error bars are used for the standard deviation (SD ± 2) of duplicate samples.

Figure 4.10. The total antioxidant capacity of GABA enriched yogurt samples.

On the other hand, total antioxidant capacities of yogurt samples (GBR20 and GBR40) have non-significantly increased as compared to control yogurt (CTR-Y) but decreased for standard GABA enriched yogurt (CTR-G). The addition of

standard GABA has increased the pH of the milk sample. Therefore, after the same period of fermentation, CTR-G yogurt has higher pH (4.72) than the control CTR-Y (4.56) and showing that fermentation was not completed for CTR-G type yogurt.

Milk casein is an acid sensitive protein, where low pH is necessary for its breakdown and formation of bioactive peptides. In our study, biosynthesis of antioxidant peptide became limited because of delay in fermentation. So, this phenomenon might explain the reduction of antioxidant capacity in CTR-G yogurt.

However, GBR crude extracts consist of many water-soluble polyphenols and reducing sugars, which improved the antioxidant capacity [148] and acidity of fortified yogurt samples, respectively. Polyphenols (ferulic and ρ-coumaric acids) in brown rice are well-known compounds for delivering antioxidant capacity to GBR and their extraction increased with water hydrolysis during germination [149]. In a study, highest antioxidant capacity was reported in yogurt produced with 3% rice bran flour [139].

4.3.8. Sensory Evaluation of Yogurt Samples

Only three types of yogurt samples (control CTY-Y, GBR20, and GBR40) were evaluated by 12 panelists after 1 day of storage at 4°C. As second control CTR-G was prepared by using standard GABA, therefore it was not included in sensory analyses. Different sensorial aspects like appearance, taste/flavor, color and overall acceptability were determined by using a 9-point Hedonic scale. In this scale,1 to 9 numbers were used for increased likeness in an ascending order. Average sensory results were represented in radar type graph as shown in Figure 4.11.

As compared to control CTR-Y yogurt, after-taste (cooked flavor) and browning color have been developed in fortified yogurt with crude extract of 40 g GBR. The taste and color development were negligible in fortified yogurt with crude extract of 20 g GBR. And the yellowish color of GBR20 yogurt gave the impression of full-fat conventional yogurt as reported by some panelists. Also, GBR20 yogurt sample has been rewarded with highest scores for its coloring parameter (>8) and high overall acceptability of this sample mainly influenced by the color too.

The cooked flavor and brownish color of yogurt samples depend on Maillard reaction products in crude GABA extracts, which were produced during high-temperature sterilization (121°C for 15 min).

Control yogurt (CTR-Y) was awarded by 7-9 points, which shows its higher acceptability. In terms of all characteristics, GBR40 yogurt has been given by lowest points (4-5) as compared to other types of yogurt. Yet, this yogurt sample was not completely rejected, by some panelists. On the other hand, yogurt with crude extract of 20 g GBR was selected as most favorable yogurt. Therefore, GBR20 functional yogurt has been suggested as a suitable replacement of conventional yogurt on the base of organoleptic properties.

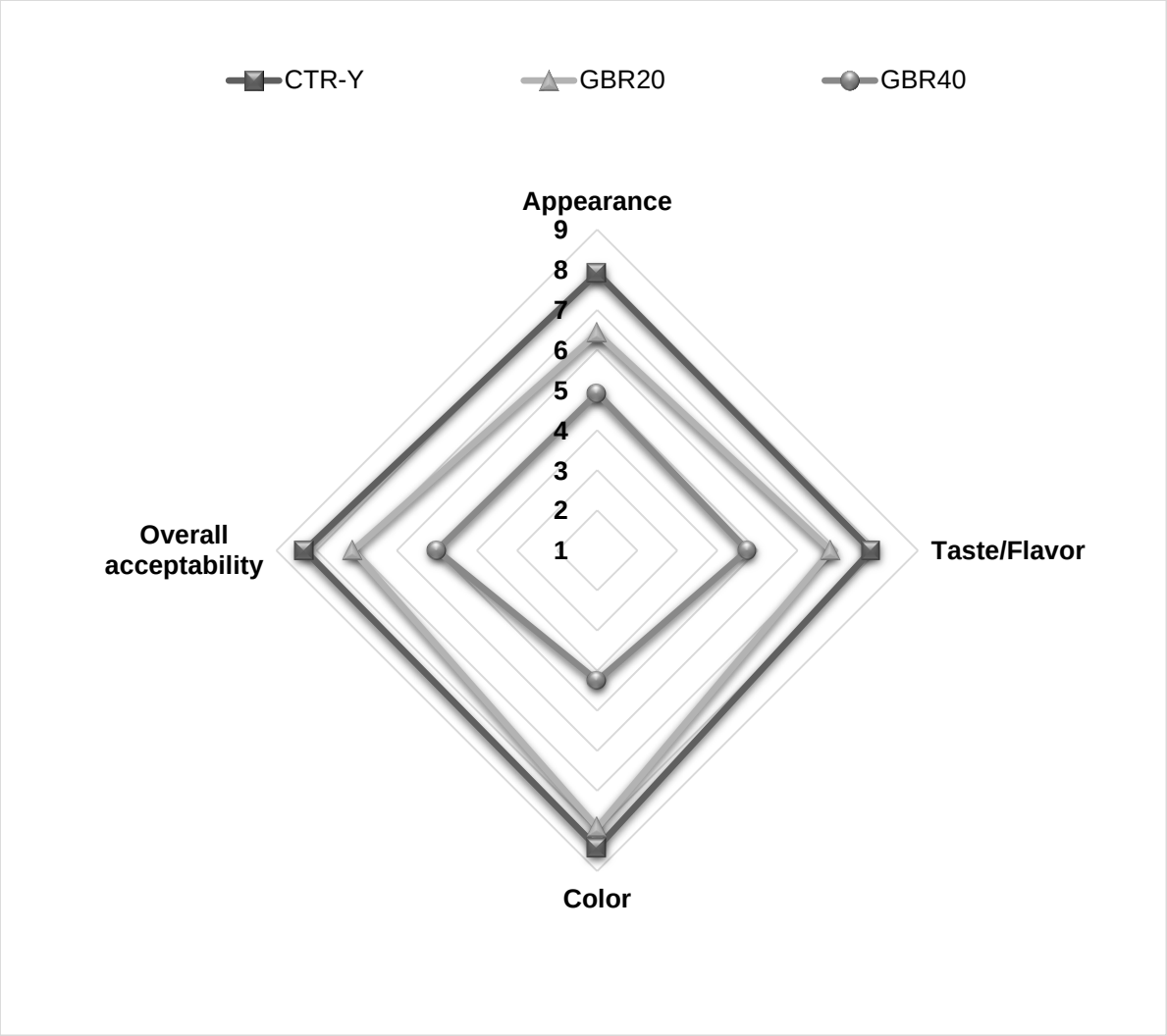


Figure 4.11. Sensory evaluation of control and GABA fortified yogurt samples.

5. CONCLUSION AND RECOMMENDATION

In this study, a new type of functional yogurt was developed. The production of yogurt samples was achieved according to Turkish standard, by using *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Functional yogurt samples were fortified by GABA containing crude extracts. After 84 hours of germination, crude GBR extracts were prepared by using water based extraction method. About 12 and 26 mg GABA contents were determined by HPLC in crude extracts obtained from 20 and 40 g of GBR, respectively.

In order to increase the GABA content, germination of brown rice was carried out at different hours. Then, GABA quantity, total γ-oryzanol and antioxidant capacity of brown rice (before and after germination) were determined after oven drying (30°C) and grinding. A positive trend was observed for GABA content by germination until 84 hours, where GABA was increased 14 times as compared to non-germinated rice. A little rise in total antioxidant capacity of GBR was observed with the germination. Whereas, γ-oryzanol contents did not change with germination significantly.

Turkish set type yogurt (control) does not contain any detectable amount GABA after fermentation. Therefore, yogurt sample with standard GABA was prepared and used as a second control in order to make comparison of GABA quantities in fortified yogurt. It was noticed that GABA content in all yogurt samples has decreased by the fermentation process, however after fermentation, GABA amount was preserved throughout the storage period.

Later, physicochemical analyses, water holding capacity, texture analyses, total antioxidant capacity and sensory evaluation were also determined for all yogurt samples during 21 days of storage at 4°C. WHC for fortified yogurt samples were significantly decreased as compared to control yogurt (CTR-Y). However, it was found that WHC of all yogurt samples increased for 14 days of storage and reduced minutely after 21 days. Same phenomena were observed for textural components (hardness and gumminess). On the other hand, cohesiveness and springiness were improved non-significantly for GABA fortified yogurt samples as compared to control yogurt. The total antioxidant capacity was also measured, and no significant change was observed in all yogurt samples.

A sensory evaluation survey was also conducted for Turkish type yogurt and GABA fortified yogurt samples produced by GBR extracts. The GABA enriched functional yogurt with crude extract of 20 g GBR was highly recommended by the panelists as the replacement for control yogurt.

In our findings, only one portion (100 g) of GABA fortified yogurt with crude extract of 20 g GBR can deliver more than 10 mg of GABA, which is enough for the treatment of individuals with mild hypertension.

In the end, following recommendations were suggested for future work;

- Use of caramel flavor for masking impaired tastes in yogurt with higher GBR extracts is highly recommended.
- The mixing of fruit pulps is another way of quality improvement for GBR containing stirred type yogurt, similar to *Activia*® type yogurt.
- Instead of autoclaving the crude extracts for sterilization, micro-filtration based methods are highly suggested for microbial reduction. As high temperature causes the production of brownish Maillard reaction products, which result in the development of undesirable color and taste. Therefore, yogurt samples prepared with a crude extract of more than 20 g GBR were less acceptable.
- Manufacturing of functional yogurts can also be advised without sterilization, by adding GABA containing extracts in milk before pasteurization.
- Bioavailability of GABA in human gastrointestinal tract should be investigated with the consumption of commercial GABA fortified yogurt.

In future, we will extend this research to next level, in which germination process will be optimized for other cereal products. Furthermore, a low price cereal based yogurt will be introduced as a functional food.

REFERENCES

[1] da Silva, B. V.; Barreira, J. C.; Oliveira, M. B. P., Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies, *Trends in Food Science & Technology*, 50, 144-158, **2016**.

[2] Chen, L.; Zhao, H.; Zhang, C.; Lu, Y.; Zhu, X.; Lu, Z., γ-Aminobutyric acid-rich yogurt fermented by Streptococcus salivarius subsp. thermophiles fmb5 apprars to have anti-diabetic effect on streptozotocin-induced diabetic mice, *Journal of Functional Foods*, 20, 267-275, **2016**.

[3] Vergari, F.; Tibuzzi, A.; Basile, G., An Overview of the Functional Food Market: From Marketing Issues and Commercial Players to Future Demand from Life in Space. *Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors*, (eds: Giardi, M. T.; Rea, G.; Berra, B., Springer US: Boston, MA, 308-321, **2010**.

[4] Wang, Y.; Liu, M.; Zhao, L.; Qiu, Y.; Zhuang, Y., Interactions of γ-aminobutyric acid and whey proteins/caseins during fortified milk production, *RSC Advances*, 5, 91235-91245, **2015**.

[5] Nejati, F.; Rizzello, C. G.; Di Cagno, R.; Sheikh-Zeinoddin, M.; Diviccaro, A.; Minervini, F.; Gobbetti, M., Manufacture of a functional fermented milk enriched of Angiotensin-I Converting Enzyme (ACE)-inhibitory peptides and γ-amino butyric acid (GABA), *LWT - Food Science and Technology*, 51, 183-189, **2013**.

[6] Khan, M. I.; Arshad, M. S.; Anjum, F. M.; Sameen, A.; Gill, W. T., Meat as a functional food with special reference to probiotic sausages, *Food Research International*, 44, 3125-3133, **2011**.

[7] Al-Muzafar, H. M.; Amin, K. A., Efficacy of functional foods mixture in improving hypercholesterolemia, inflammatory and endothelial dysfunction biomarkers-induced by high cholesterol diet, *Lipids in health and disease*, 16, 194, **2017**.

[8] Mirmiran, P.; Bahadoran, Z.; Azizi, F., Functional foods-based diet as a novel dietary approach for management of type 2 diabetes and its complications: A review, *World journal of diabetes*, 5, 267, **2014**.

[9] Pandey, K. B.; Rizvi, S. I., Plant polyphenols as dietary antioxidants in human health and disease, *Oxidative medicine and cellular longevity*, 2, 270-278, **2009**.

[10] Wu, F.; Yang, N.; Touré, A.; Jin, Z.; Xu, X., Germinated brown rice and its role in human health, *Critical reviews in food science and nutrition*, 53, 451-463, **2013**.

[11] Juliano, B. O., RICE A2 - Caballero, Benjamin. *Encyclopedia of Food Sciences and Nutrition (Second Edition)*, (eds: Academic Press: Oxford, 4995-5001, **2003**.

[12] Verma, D. K.; Srivastav, P. P., Proximate Composition, Mineral Content and Fatty Acids Analyses of Aromatic and Non-Aromatic Indian Rice, *Rice Science*, 24, 21-31, **2017**.

[13] Hata, S.; Wiboonsirikul, J.; Maeda, A.; Kimura, Y.; Adachi, S., Extraction of defatted rice bran by subcritical water treatment, *Biochemical Engineering Journal*, 40, 44-53, **2008**.

[14] Varanyanond, W.; Tungtrakul, P.; Surojanametakul, V.; Watanasiritham, L.; Luxiang, W., Effect of water soaking on gamma-aminobutyric acid (GABA) in germ of different Thai rice varieties, *Kasetsart J.(Nat. Sci.)*, 39, 411-415, **2005**.

[15] Tian, S.; Nakamura, K.; Kayahara, H., Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice, *Journal of agricultural and food chemistry*, 52, 4808-4813, **2004**.

[16] Saunders, R., Rice bran: composition and potential food uses, *Food Reviews International*, 1, 465-495, **1985**.

[17] Cáceres, P. J.; Peñas, E.; Martinez-Villaluenga, C.; Amigo, L.; Frias, J., Enhancement of biologically active compounds in germinated brown rice and the effect of sun-drying, *Journal of Cereal Science*, 73, 1-9, **2017**.

[18] Oko, A.; Ubi, B.; Efisue, A.; Dambaba, N., Comparative analysis of the chemical nutrient composition of selected local and newly introduced rice varieties grown in Ebonyi State of Nigeria, *International Journal of Agriculture and Forestry*, 2, 16-23, **2012**.

[19] Izadi, V.; Azadbakht, L., Is there any association between rice consumption and some of the cardiovascular diseases risk factors? A systematic review, *ARYA atherosclerosis*, 11, 109, **2015**.

[20] Cáceres, P. J.; Martínez-Villaluenga, C.; Amigo, L.; Frias, J., Maximising the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions, *Food Chemistry*, 152, 407-414, **2014**.

[21] Ito, S.; Ishikawa, Y. In Marketing of value-added rice products in Japan: germinated brown rice and rice bread, *FAO rice conference Rome, Italy*, pp 12-13 **2004**.

[22] Banchuen, J. *Bio-active compounds in germinated brown rice and its application*, PhD Thesis, Prince of Songkla University Department of Food Technology, Songkhla, **2010**.

[23] Roy, P.; Orikasa, T.; Okadome, H.; Nakamura, N.; Shiina, T., Processing conditions, rice properties, health and environment, *International journal of environmental research and public health*, 8, 1957-1976, **2011**.

[24] Bewley, J. D., Seed germination and reserve mobilization, *eLS*, **2001**.

[25] Bewley, J. D.; Bradford, K. J.; Hilhorst, H. W.; Nonogaki, H., Germination. *Seeds*, (eds: Springer: 133-181, **2013**.

[26] Bewley, J. D., Seed germination and dormancy, *The plant cell*, 9, 1055, **1997**.

[27] Cho, D. H.; Lim, S. T., Germinated brown rice and its bio-functional compounds, *Food Chemistry*, 196, 259-271, **2016**.

[28] Wu, F.; Chen, H.; Yang, N.; Wang, J.; Duan, X.; Jin, Z.; Xu, X., Effect of germination time on physicochemical properties of brown rice flour and starch from different rice cultivars, *Journal of Cereal Science*, 58, 263-271, **2013**.

[29] Cao, Y.; Jia, F.; Han, Y.; Liu, Y.; Zhang, Q., Study on the optimal moisture adding rate of brown rice during germination by using segmented moisture conditioning method, *Journal of food science and technology*, 52, 6599-6606, **2015**.

[30] Patil, S. B.; Khan, M. K., Germinated brown rice as a value added rice product: A review, *Journal of food science and technology*, 48, 661-667, **2011**.

[31] Xu, J.; Zhang, H.; Guo, X.; Qian, H., The impact of germination on the characteristics of brown rice flour and starch, *Journal of the Science of Food and Agriculture*, 92, 380-387, **2012**.

[32] Moongngarm, A.; Saetung, N., Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice, *Food Chemistry*, 122, 782-788, **2010**.

[33] Oh, C. H.; Oh, S. H., Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis, *Journal of medicinal food*, 7, 19-23, **2004**.

[34] Kaosa-ard, T.; Songsermpong, S., Influence of germination time on the GABA content and physical properties of germinated brown rice, *Asian Journal of Food and Agro-Industry*, 5, 270-283, **2012**.

[35] Meeploy, M.; Deewatthanawong, R., Determination of γ-Aminobutyric Acid (GABA) in Rambutan Fruit cv. Rongrian by HPLC-ELSD and Separation of GABA from Rambutan Fruit Using Dowex 50W-X8 Column, *Journal of chromatographic science*, 54, 445-452, **2015**.

[36] Shan, Y.; Man, C. X.; Han, X.; Li, L.; Guo, Y.; Deng, Y.; Li, T.; Zhang, L. W.; Jiang, Y. J., Evaluation of improved γ-aminobutyric acid production in yogurt using Lactobacillus plantarum NDC75017, *Journal of Dairy Science*, 98, 2138-2149, **2015**.

[37] Diana, M.; Quílez, J.; Rafecas, M., Gamma-aminobutyric acid as a bioactive compound in foods: a review, *Journal of Functional Foods*, 10, 407-420, **2014**.

[38] Eamarjharn, A.; Theerakulkait, C.; Thanachasai, S., Effect of incubation time, buffer type and concentration on gamma-aminobutyric acid (GABA) production using Khao Dawk Mali 105 rice bran, *Agriculture and Natural Resources*, 50, 80-84, **2016**.

[39] Steward, F.; Thompson, J.; Dent, C., γ-Aminobutyric acid: a constituent of the potato tuber?, *Science*, 110, 439-440, **1949**.

[40] Saikusa, T.; Horino, T.; Mori, Y., Distribution of free amino acids in the rice kernel and kernel fractions and the effect of water soaking on the distribution, *Journal of agricultural and food chemistry*, 42, 1122-1125, **1994**.

[41] Banchuen, J.; Thammarutwasik, P.; Ooraikul, B.; Wuttijumnong, P.; Sirivongpaisal, P., Increasing the bio-active compounds contents by optimizing the germination conditions of southern Thai brown rice, *Songklanakarin Journal of Science and Technology*, 32, 219-230, **2010**.

[42] Lu, Z. H.; Zhang, Y.; Li, L.-T.; Curtis, R. B.; Kong, X. L.; Fulcher, R. G.; Zhang, G.; Cao, W., Inhibition of microbial growth and enrichment of γ-aminobutyric acid during germination of brown rice by electrolyzed oxidizing water, *Journal of food protection*, 73, 483-487, **2010**.

[43] Dhakal, R.; Bajpai, V. K.; Baek, K.-H., Production of GABA (γ-aminobutyric acid) by microorganisms: a review, *Brazilian Journal of Microbiology*, 43, 1230-1241, **2012**.

[44] Inoue, K.; Shirai, T.; Ochiai, H.; Kasao, M.; Hayakawa, K.; Kimura, M.; Sansawa, H., Blood-pressure-lowering effect of a novel fermented milk containing [gamma]-aminobutyric acid (GABA) in mild hypertensives, *Eur J Clin Nutr*, 57, 490-495, **2003**.

[45] Dat, L. Q.; Ngan, T. T. K.; Nu, N. T. X., Gamma-amino butyric acid (GABA) synthesis of Lactobacillus in fermentation of defatted rice bran extract, *AIP Conference Proceedings*, 1878, 020045, **2017**.

[46] Ko, C. Y.; Lin, H. T. V.; Tsai, G. J., Gamma-aminobutyric acid production in black soybean milk by Lactobacillus brevis FPA 3709 and the antidepressant effect of the fermented product on a forced swimming rat model, *Process Biochemistry*, 48, 559-568, **2013**.

[47] Pihlanto, A.; Korhonen, H., 3 - Bioactive peptides from fermented foods and health promotion A2 - Holzapfel, Wilhelm. *Advances in Fermented Foods and Beverages*, (eds: Woodhead Publishing: 39-74, **2015**.

[48] Junxia, G. X. D. J. X., Quality Control on Germinated Brown Rice Yoghurt Rich in Ferulic Acid-and γ-GABA [J], *Journal of Chinese Institute of Food Science and Technology*, 7, 021, **2011**.

[49] Kook, M. C.; Cho, S. C., Production of GABA (gamma amino butyric acid) by lactic acid bacteria, *Korean Journal for Food Science of Animal Resources*, 33, 377-389, **2013**.

[50] Xie, Z.; Xia, S.; Le, G. W., Gamma-aminobutyric acid improves oxidative stress and function of the thyroid in high-fat diet fed mice, *Journal of Functional Foods*, 8, 76-86, **2014**.

[51] Hagi, T.; Kobayashi, M.; Nomura, M., Metabolome analysis of milk fermented by γ-aminobutyric acid–producing *Lactococcus lactis*, *Journal of Dairy Science*, 99, 994-1001, **2016**.

[52] Sun, T.; Zhao, S.; Wang, H.; Cai, C.; Chen, Y.; Zhang, H., ACE-inhibitory activity and gamma-aminobutyric acid content of fermented skim milk by Lactobacillus helveticus isolated from Xinjiang koumiss in China, *European Food Research and Technology*, 228, 607-612, **2009**.

[53] Liu, C. F.; Tung, Y. T.; Wu, C. L.; Lee, B.-H.; Hsu, W.-H.; Pan, T. M., Antihypertensive effects of Lactobacillus-fermented milk orally administered to spontaneously hypertensive rats, *Journal of agricultural and food chemistry*, 59, 4537-4543, **2011**.

[54] Zhang, Q.; Xiang, J.; Zhang, L.; Zhu, X.; Evers, J.; van der Werf, W.; Duan, L., Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice, *Journal of Functional Foods*, 10, 283-291, **2014**.

[55] Kaneko, R.; Tsuchiya, T., New compound in rice bran and germ oils, *J Chem Soc Jpn*, 57, 526-529, **1954**.

[56] Srichamnong, W.; Thiyajai, P.; Charoenkiatkul, S., Conventional steaming retains tocols and γ-oryzanol better than boiling and frying in the jasmine rice variety Khao dok mali 105, *Food Chemistry*, 191, 113-119, **2016**.

[57] Banchuen, J.; Thammarutwasik, P.; Ooraikul, B.; Wuttijumnong, P.; Sirivongpaisal, P., Effect of germinating processes on bioactive component of Sangyod Muang Phatthalung rice, *Thai Journal of Agricultural Science*, 42, 191-199, **2009**.

[58] Lemus, C.; Angelis, A.; Halabalaki, M.; Skaltsounis, A. L., Chapter 32 - γ-Oryzanol: An Attractive Bioactive Component from Rice Bran A2 - Watson, Ronald Ross. *Wheat and Rice in Disease Prevention and Health*, (eds: Preedy, V. R.; Zibadi, S., Academic Press: San Diego, 409-430, **2014**.

[59] Jay, J.; Loessner, M.; Golden, D., Milk, Fermentation, and Fermented and Nonfermented Dairy Products. *Modern Food Microbiology*, (eds: Springer US: Boston, MA, 149-173, **2005**.

[60] Codex Alimentarius Commission, *Standard for fermented milks*, CODEX STAN 243, **2003**.

[61] Aghababaie, M.; Khanahmadi, M.; Beheshti, M., Developing a kinetic model for co-culture of yogurt starter bacteria growth in pH controlled batch fermentation, *Journal of Food Engineering*, 166, 72-79, **2015**.

[62] Yıldız, F., Overview of yogurt and other fermented dairy products, *Development and Manufacture of Yogurt and Other Functional Dairy Products*, 1-46, **2010**.

[63] Elgasim, E. A. *The Use of Natural Plant Stabilizers Extracts in the Production of Yoghurt*, Master Thesis, University of Khartoum Department of Food Science and Technology, Khartoum, **2015**.

[64] Nor-Khaizura, M.-A.-R.; Flint, S. H.; McCarthy, O. J.; Palmer, J. S.; Golding, M.; Jaworska, A., Development of made-in-transit set culture yoghurt: effect of increasing the concentration of reconstituted skim milk as the milk base, *International Journal of Food Science & Technology*, 47, 579-584, **2012**.

[65] Akın, N., *Modern yoğurt bilimi ve teknolojisi*. Selçuk Üniversitesi Ziraat Fakültesi Gıda Mühendisliği Bölümü: Konya, Turkey;**2006**.

[66] Tamime, A. Y.; Robinson, R. K., *Tamime and Robinson's yoghurt: science and technology*. Elsevier;**2007**.

[67] Weerathilake, W.; Rasika, D.; Ruwanmali, J.; Munasinghe, M., The evolution, processing, varieties and health benefits of yogurt, *International Journal of Scientific and Research Publications*, 4, 1-10, **2014**.

[68] Robinson, R. K.; Lucey, J. A.; Tamime, A. Y., Manufacture of Yoghurt. *Fermented Milks*, (eds: Blackwell Publishing Ltd: 53-75, **2007**.

[69] Tekinsen, O., *Süt ürünleri teknolojisi*. Selcuk Universitesi Basimevi: Konya, Turkey, Vol. 1, p 86;**2000**.

[70] Walstra, P.; Walstra, P.; Wouters, J. T.; Geurts, T. J., *Dairy science and technology*. Second ed.; CRC press;**2006**.

[71] Lucey, J. A., Cultured dairy products: an overview of their gelation and texture properties, *International journal of dairy technology*, 57, 77-84, **2004**.

[72] Lucey, J., Formation, structural properties and rheology of acid-coagulated milk gels, *Cheese: Chemistry, physics and microbiology*, 1, 105-122, **2004**.

[73] Phadungath, C., The mechanism and properties of acid-coagulated milk gels, *Songklanakarin J. Sci. Technol*, 27, 433-448, **2005**.

[74] Lee, W.; Lucey, J., Formation and physical properties of yogurt, *Asian-Australasian Journal of Animal Sciences*, 23, 1127-1136, **2010**.

[75] Routray, W.; Mishra, H. N., Scientific and technical aspects of yogurt aroma and taste: a review, *Comprehensive Reviews in Food Science and Food Safety*, 10, 208-220, **2011**.

[76] Cheng, H., Volatile flavor compounds in yogurt: a review, *Critical reviews in food science and nutrition*, 50, 938-950, **2010**.

[77] Sodini, I.; Remeuf, F.; Haddad, S.; Corrieu, G., The relative effect of milk base, starter, and process on yogurt texture: a review, *Critical reviews in food science and nutrition*, 44, 113-137, **2004**.

[78] Vanishree, S.; Kammar, M.; Nidoni, U., Development and evaluation of pearl millet based novel health drink, *Advances in Life Sciences*, 5, 5483-5486, **2016**.

[79] Nollet, L. M.; Toldrá, F., *Sensory analysis of foods of animal origin*. CRC Press;**2010**.

[80] do Espírito Santo, A. P.; Perego, P.; Converti, A.; Oliveira, M. N., Influence of milk type and addition of passion fruit peel powder on fermentation kinetics, texture profile and bacterial viability in probiotic yoghurts, *LWT - Food Science and Technology*, 47, 393-399, **2012**.

[81] Karam, M. C.; Gaiani, C.; Hosri, C.; Burgain, J.; Scher, J., Effect of dairy powders fortification on yogurt textural and sensorial properties: a review, *Journal of dairy research*, 80, 400-409, **2013**.

[82] Çelik, E. S. *Determination of aroma compounds and exopolysaccharides formation by lactic acid bacteri isolated from traditional yogurts*, Master Thesis, Izmir Institute of Technology Departmant of Biotecnology and Bioengineering, Izmir, **2007**.

[83] Lucey, J. A., The relationship between rheological parameters and whey separation in milk gels, *Food Hydrocolloids*, 15, 603-608, **2001**.

[84] Mckinley, M. C., The nutrition and health benefits of yoghurt, *International journal of dairy technology*, 58, 1-12, **2005**.

[85] De Paula, J. A.; Carmuega, E.; Weill, R., Effect of the ingestion of a symbiotic yogurt on the bowel habits of women with functional constipation, *Acta Gastroenterológica Latinoamericana*, 38, **2008**.

[86] Lourens-Hattingh, A.; Viljoen, B. C., Yogurt as probiotic carrier food, *International Dairy Journal*, 11, 1-17, **2001**.

[87] do Espírito Santo, A. P.; Cartolano, N. S.; Silva, T. F.; Soares, F. A. S. M.; Gioielli, L. A.; Perego, P.; Converti, A.; Oliveira, M. N., Fibers from fruit by-products enhance probiotic viability and fatty acid profile and increase CLA content in yoghurts, *International Journal of Food Microbiology*, 154, 135-144, **2012**.

[88] Espírito-Santo, A. P.; Lagazzo, A.; Sousa, A. L. O. P.; Perego, P.; Converti, A.; Oliveira, M. N., Rheology, spontaneous whey separation, microstructure and sensorial characteristics of probiotic yoghurts enriched with passion fruit fiber, *Food Research International*, 50, 224-231, **2013**.

[89] Perina, N. P.; Granato, D.; Hirota, C.; Cruz, A. G.; Bogsan, C. S. B.; Oliveira, M. N., Effect of vegetal-oil emulsion and passion fruit peel-powder on sensory acceptance of functional yogurt, *Food Research International*, 70, 134-141, **2015**.

[90] Sah, B. N. P.; Vasiljevic, T.; McKechnie, S.; Donkor, O. N., Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage, *LWT - Food Science and Technology*, 65, 978-986, **2016**.

[91] Dal Bello, B.; Torri, L.; Piochi, M.; Zeppa, G., Healthy yogurt fortified with n-3 fatty acids from vegetable sources, *Journal of Dairy Science*, 98, 8375-8385, **2015**.

[92] Rognlien, M.; Duncan, S.; O'Keefe, S.; Eigel, W., Consumer perception and sensory effect of oxidation in savory-flavored yogurt enriched with n-3 lipids, *Journal of Dairy Science*, 95, 1690-1698, **2012**.

[93] Georgakouli, K.; Mpesios, A.; Kouretas, D.; Petrotos, K.; Mitsagga, C.; Giavasis, I.; Jamurtas, A. Z., The Effects of an Olive Fruit Polyphenol-Enriched Yogurt on Body Composition, Blood Redox Status, Physiological and Metabolic Parameters and Yogurt Microflora, *Nutrients*, 8, 344, **2016**.

[94] Petrotos, K. B.; Karkanta, F. K.; Gkoutsidis, P. E.; Giavasis, I.; Papatheodorou, K. N.; Ntontos, A. C., Production of novel bioactive yogurt enriched with olive fruit polyphenols, *World Academy of Science, Engineering and Technology*, 64, 867-872, **2012**.

[95] Karaaslan, M.; Ozden, M.; Vardin, H.; Turkoglu, H., Phenolic fortification of yogurt using grape and callus extracts, *LWT - Food Science and Technology*, 44, 1065-1072, **2011**.

[96] Emirdağı, H. *Textural evaluation possibilities of resveratrol in yogurt*, Master Thesis, Hacettepe University Department of Food Engineering, Ankara, **2014**.

[97] Park, K.; Oh, S., Production and characterization of GABA rice yogurt, *Food Science and Biotechnology*, 14, 518, **2005**.

[98] Park, K. B.; Oh, S. H., Production of yogurt with enhanced levels of gamma-aminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract, *Bioresource Technology*, 98, 1675-1679, **2007**.

[99] Kim, M.-H.; Ahn, S.-I.; Lim, C.-M.; Jhoo, J.-W.; Kim, G.-Y., Effects of Germinated Brown Rice Addition on the Flavor and Functionality of Yogurt, *Korean Journal for Food Science of Animal Resources*, 36, 508, **2016**.

[100] Anawachkul, M.; Jiamyangyuen, S., The Study of GABA Content and Development of GABA Enriched Yogurt from Germinated Red Rice (Munpoo rice), *Kasetsart Journal - Natural Science*, 43, 224-231, **2009**.

[101] Liu, M.; Wang, Y.; Jiang, L.; Xia, Q.; Qiu, Y.; Fan, L.; Zhou, J.; Zhao, L., Effect of γ-aminobutyric acid on the physicochemical, rheological and sensory properties of yoghurt, *International journal of dairy technology*, 68, 503-510, **2015**.

[102] Linares, D. M.; O'Callaghan, T. F.; O'Connor, P. M.; Ross, R. P.; Stanton, C., Streptococcus thermophilus APC151 Strain Is Suitable for the Manufacture of Naturally GABA-Enriched Bioactive Yogurt, *Frontiers in Microbiology*, 7, **2016**.

[103] Jannoey, P.; Niamsup, H.; Lumyong, S.; Suzuki, T.; Katayama, T.; Chairote, G., Comparison of gamma-aminobutyric acid production in Thai rice grains, *World Journal of Microbiology and Biotechnology*, 26, 257-263, **2010**.

[104] AACC, A. M. C., *Approved methods of the American association of cereal chemists*. Amer Assn of Cereal Chemists: Vol. 1;**2000**.

[105] Soi-ampornkul, R.; Junnu, S.; Kanyok, S.; Liammongkolkul, S.; Katanyoo, W.; Umpornsirirat, S., Antioxidative and neuroprotective activities of the pre-germinated brown rice extract, *Food and Nutrition Sciences*, 3, 135, **2012**.

[106] AOAC, *Official methods of analysis of AOAC International*. Method 947.05. Intl. 17th ed.; Association of Official Analytical Chemists, Arlington, VA, USA;**2000**.

[107] Jannoey, P.; Niamsup, H.; Lumyong, S.; Tajima, S.; Nomura, M.; Chairote, G., γ-aminobutyric acid (GABA) accumulations in rice during germination, *Chiang Mai J. Sci*, 37, 124-133, **2010**.

[108] Castillo, M.; Castells, R., Procedure for the determination of biogenic amines by derivatization with dabsyl chloride and reversed phase liquid chromatography, *Chromatographia*, 54, 711-716, **2001**.

[109] Jeng, T. L.; Ho, P. T.; Shih, Y. J.; Lai, C. C.; Wu, M. T.; Sung, J. M., Comparisons of protein, lipid, phenolics, γ-oryzanol, vitamin E, and mineral contents in bran layer of sodium azide-induced red rice mutants, *Journal of the Science of Food and Agriculture*, 91, 1459-1465, **2011**.

[110] Sahan, N.; Yasar, K.; Hayaloglu, A. A., Physical, chemical and flavour quality of non-fat yogurt as affected by a β-glucan hydrocolloidal composite during storage, *Food Hydrocolloids*, 22, 1291-1297, **2008**.

[111] Najgebauer-Lejko, D.; Żmudziński, D.; Ptaszek, A.; Socha, R., Textural properties of yogurts with green tea and Pu-erh tea additive, *International Journal of Food Science & Technology*, 49, 1149-1158, **2014**.

[112] Serpen, A.; Gökmen, V.; Pellegrini, N.; Fogliano, V., Direct measurement of the total antioxidant capacity of cereal products, *Journal of Cereal Science*, 48, 816-820, **2008**.

[113] Peryam, D. R.; Girardot, N. F., Advanced taste-test method, *Food Engineering*, 24, 58-61, **1952**.

[114] Wu, P.; Deng, R.; Wu, X.; Wang, Y.; Dong, Z.; Dhital, S.; Chen, X. D., In vitro gastric digestion of cooked white and brown rice using a dynamic rat stomach model, *Food Chemistry*, 237, 1065-1072, **2017**.

[115] Somaratne, G. M.; Prasantha, B. D. R.; Dunuwila, G. R.; Chandrasekara, A.; Wijesinghe, D. G. N. G.; Gunasekara, D. C. S., Effect of polishing on glycemic index and antioxidant properties of red and white basmati rice, *Food Chemistry*, 237, 716-723, **2017**.

[116] Zhou, Z.; Chen, X.; Zhang, M.; Blanchard, C., Phenolics, flavonoids, proanthocyanidin and antioxidant activity of brown rice with different pericarp colors following storage, *Journal of Stored Products Research*, 59, 120-125, **2014**.

[117] Wei, Y.; Shohag, M.; Wang, Y.; Lu, L.; Wu, C.; Yang, X., Effect of zinc sulfate fortification in germinated brown rice on seed zinc concentration, bioavailability, and seed germination, *Journal of agricultural and food chemistry*, 60, 1871-1879, **2012**.

[118] Turan, A. *The effect of germinated brown rice cake production on bioactive compounds*, Master Thesis, Hacettepe University Department of Food Engineering, Ankara, **2013**.

[119] Bari, M. L.; Enomoto, K.; Nei, D.; Kawamoto, S., Development of Effective Seed Decontamination Technology to Inactivate Pathogens on Mung Bean Seeds and Its Practical Application in Japan, *Japan Agricultural Research Quarterly: JARQ*, 45, 153-161, **2011**.

[120] Imam, M. U.; Musa, S. N. A.; Azmi, N. H.; Ismail, M., Effects of white rice, brown rice and germinated brown rice on antioxidant status of type 2 diabetic rats, *International journal of molecular sciences*, 13, 12952-12969, **2012**.

[121] Liu, R.; He, X.; Shi, J.; Nirasawa, S.; Tatsumi, E.; Li, L.; Liu, H., The effect of electrolyzed water on decontamination, germination and γ-aminobutyric acid accumulation of brown rice, *Food Control*, 33, 1-5, **2013**.

[122] Kunze, O. R., Effect of Drying on Grain Quality--Moisture Readsorption Causes Fissured Rice Grains, *Agricultural Engineering International: CIGR Journal*, **2008**.

[123] Roohinejad, S.; Omidizadeh, A.; Mirhosseini, H.; Saari, N.; Mustafa, S.; Meor Hussin, A. S.; Hamid, A.; Abd Manap, M. Y., Effect of pre-germination time on amino acid profile and gamma amino butyric acid (GABA) contents in different varieties of Malaysian brown rice, *International Journal of Food Properties*, 14, 1386-1399, **2011**.

[124] Choi, I. D.; Kim, D. S.; Son, J. R.; Yang, C. I.; Chun, J. Y.; Kim, K. J., Physico-chemical properties of giant embryo brown rice (Keunnunbyeo), *Journal of Applied Biological Chemistry*, 49, 95-100, **2006**.

[125] Ohtsubo, K. i.; Suzuki, K.; Yasui, Y.; Kasumi, T., Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder, *Journal of food composition and analysis*, 18, 303-316, **2005**.

[126] Kim, H. Y.; Hwang, I. G.; Kim, T. M.; Woo, K. S.; Park, D. S.; Kim, J. H.; Kim, D. J.; Lee, J.; Lee, Y. R.; Jeong, H. S., Chemical and functional components in different parts of rough rice (Oryza sativa L.) before and after germination, *Food Chemistry*, 134, 288-293, **2012**.

[127] Watchararparpaiboon, W.; Laohakunjit, N.; Kerdchoechuen, O., An Improved Process for High Quality and Nutrition of Brown Rice Production, *Food Science and Technology International*, 16, 147-158, **2010**.

[128] Gut, H.; Dominici, P.; Pilati, S.; Astegno, A.; Petoukhov, M. V.; Svergun, D. I.; Grütter, M. G.; Capitani, G., A Common Structural Basis for pH- and Calmodulin-mediated Regulation in Plant Glutamate Decarboxylase, *Journal of Molecular Biology*, 392, 334-351, **2009**.

[129] Cornejo, F.; Caceres, P. J.; Martínez-Villaluenga, C.; Rosell, C. M.; Frias, J., Effects of germination on the nutritive value and bioactive compounds of brown rice breads, *Food Chemistry*, 173, 298-304, **2015**.

[130] Moongngarm, A.; Khomphiphatkul, E., Germination time dependence of bioactive compounds and antioxidant activity in germinated rough rice (Oryza sativa L.), *American Journal of Applied Sciences*, 8, 15, **2011**.

[131] Kiing, C.; Yiu, P. H.; Rajan, A.; Wong, S.-C., Effect of germination on γ-oryzanol content of selected Sarawak rice cultivars, *American Journal of Applied Sciences*, 6, 1658, **2009**.

[132] He, D.; Han, C.; Yao, J.; Shen, S.; Yang, P., Constructing the metabolic and regulatory pathways in germinating rice seeds through proteomic approach, *Proteomics*, 11, 2693-2713, **2011**.

[133] Ismail, M.; Al-Naqeeb, G.; Mamat, W. A. A. b.; Ahmad, Z., Gamma-oryzanol rich fraction regulates the expression of antioxidant and oxidative stress related genes in stressed rat's liver, *Nutrition & Metabolism*, 7, 23-23, **2010**.

[134] Wang, Y.; Liu, M.; Zhao, L.; Qiu, Y.; Zhuang, Y., Influence of processing conditions on reducing γ-aminobutyric acid content during fortified milk production, *Food Research International*, 72, 215-222, **2015**.

[135] Tamime, A. Y.; Robinson, R. K., 6 - Microbiology of yoghurt and related starter cultures. *Tamime and Robinson's Yoghurt (Third Edition)*, (eds: Woodhead Publishing: 468-534, **2007**.

[136] Ando, A.; Nakamura, T., Prevention of GABA reduction during dough fermentation using a baker's yeast dal81 mutant, *Journal of bioscience and bioengineering*, 122, 441-445, **2016**.

[137] Hue, C.; Gunata, Z.; Breysse, A.; Davrieux, F.; Boulanger, R.; Sauvage, F. X., Impact of fermentation on nitrogenous compounds of cocoa beans (*Theobroma cacao L.*) from various origins, *Food Chemistry*, 192, 958-964, **2016**.

[138] Watanabe, Y.; Hayakawa, K.; Ueno, H., Effects of co-culturing LAB on GABA production, *J. Biol. Macromol*, 11, 3-13, **2011**.

[139] Demirci, T.; Aktaş, K.; Sözeri, D.; Öztürk, H. İ.; Akın, N., Rice bran improve probiotic viability in yoghurt and provide added antioxidative benefits, *Journal of Functional Foods*, 36, 396-403, **2017**.

[140] Coda, R.; Lanera, A.; Trani, A.; Gobbetti, M.; Di Cagno, R., Yogurt-like beverages made of a mixture of cereals, soy and grape must: Microbiology, texture, nutritional and sensory properties, *International Journal of Food Microbiology*, 155, 120-127, **2012**.

[141] Christensen, L. M., Evaluation of textural properties of cooked beef batters, **2012**.

[142] Ozcan, T. In Determination of yogurt quality by using rheological and textural parameters, *2013 2nd International Conference on Nutrition and Food Sciences-ICNFS*, pp 27-28 **2013**.

[143] El-Gammal, R. E.; Abdel-Aziz, M.; Darwish, M., Utilization of Aqueous Extract of Moringa oleifera for Production of Functional Yogurt, *Mansoura Journal of Food and Dairy Sciences*, 8, 45-53, **2017**.

[144] Aloğlu, H. Ş.; Öner, Z., Determination of antioxidant activity of bioactive peptide fractions obtained from yogurt, *Journal of Dairy Science*, 94, 5305-5314, **2011**.

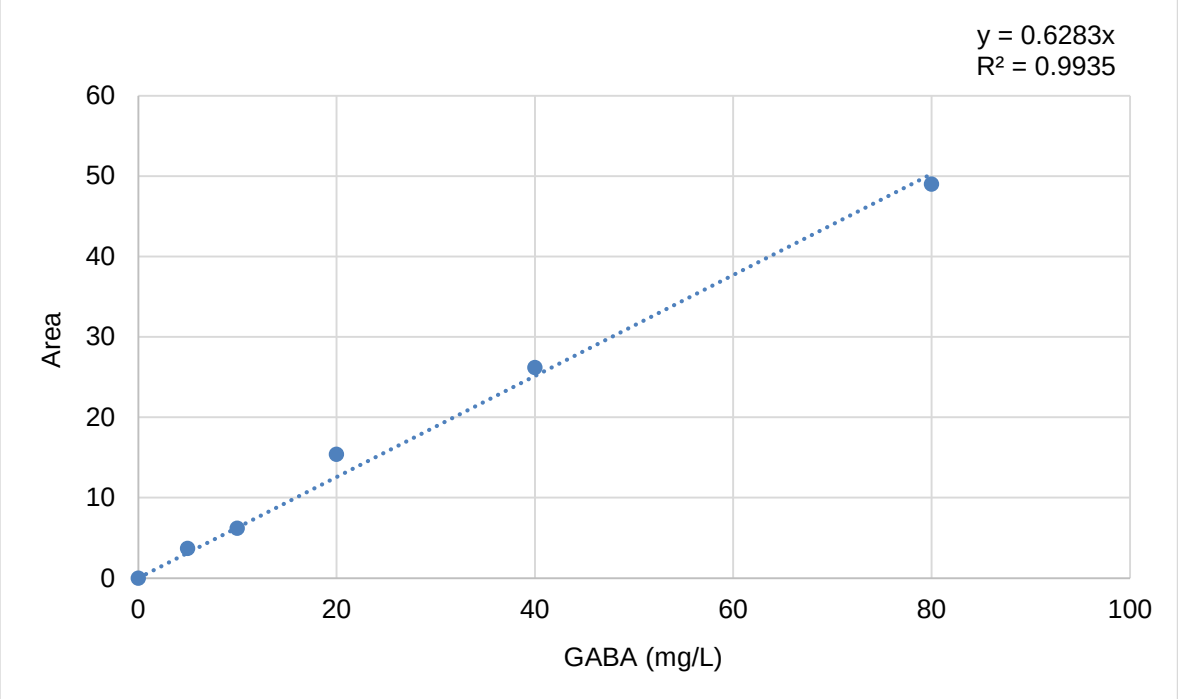
[145] Citta, A.; Folda, A.; Scalcon, V.; Scutari, G.; Bindoli, A.; Bellamio, M.; Feller, E.; Rigobello, M. P., Oxidative changes in lipids, proteins, and antioxidants in yogurt during the shelf life, *Food Science & Nutrition*, 5, 1079-1087, **2017**.

[146] Muniandy, P.; Shori, A. B.; Baba, A. S., Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage, *Food Packaging and Shelf Life*, 8, 1-8, **2016**.

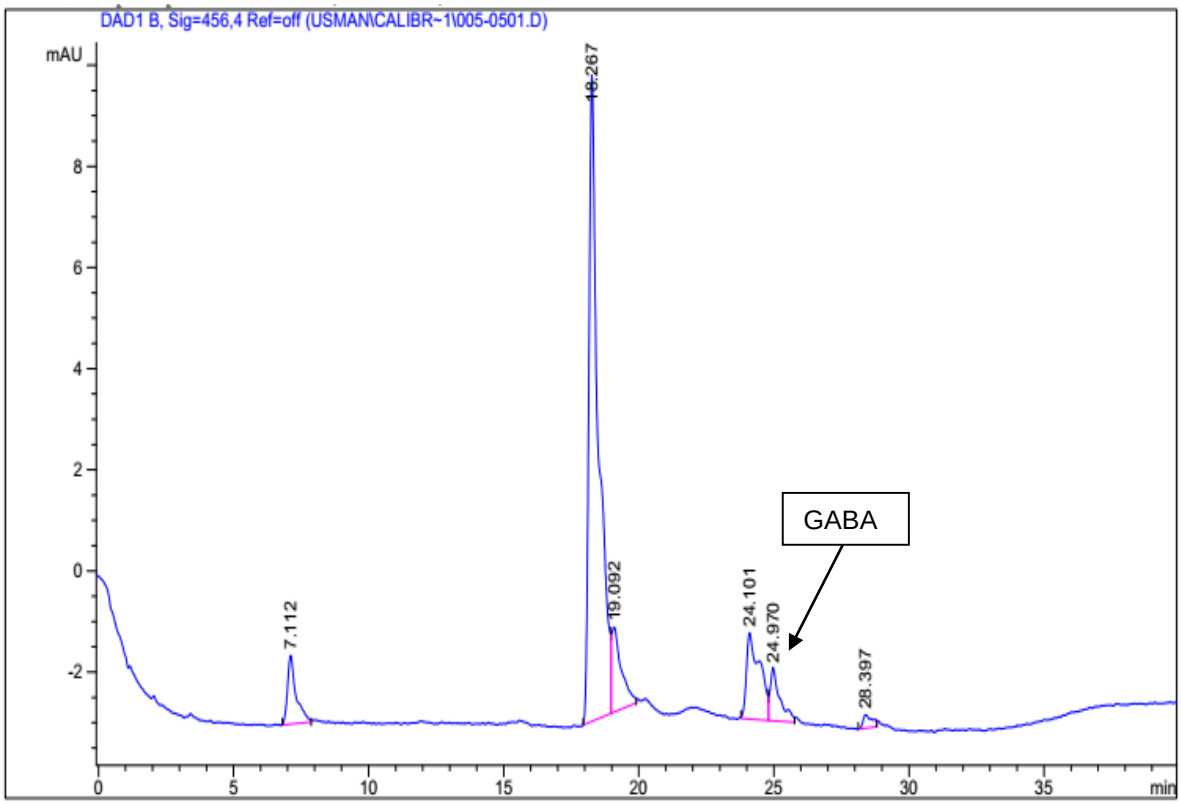
[147] Taha, S.; El Abd, M.; De Gobba, C.; Abdel-Hamid, M.; Khalil, E.; Hassan, D., Antioxidant and antibacterial activities of bioactive peptides in buffalo's yoghurt fermented with different starter cultures, *Food Science and Biotechnology*, 1-8, **2017**.

ANNEX

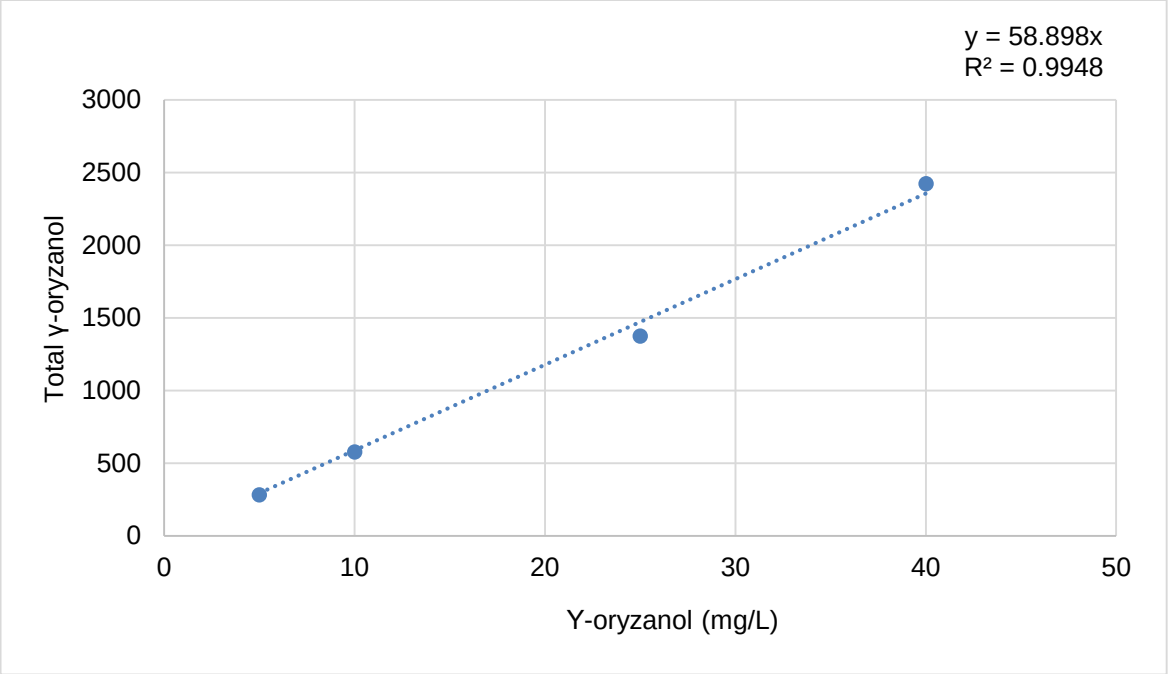
Annex 1. The slope of calibration for GABA through HPLC**.**



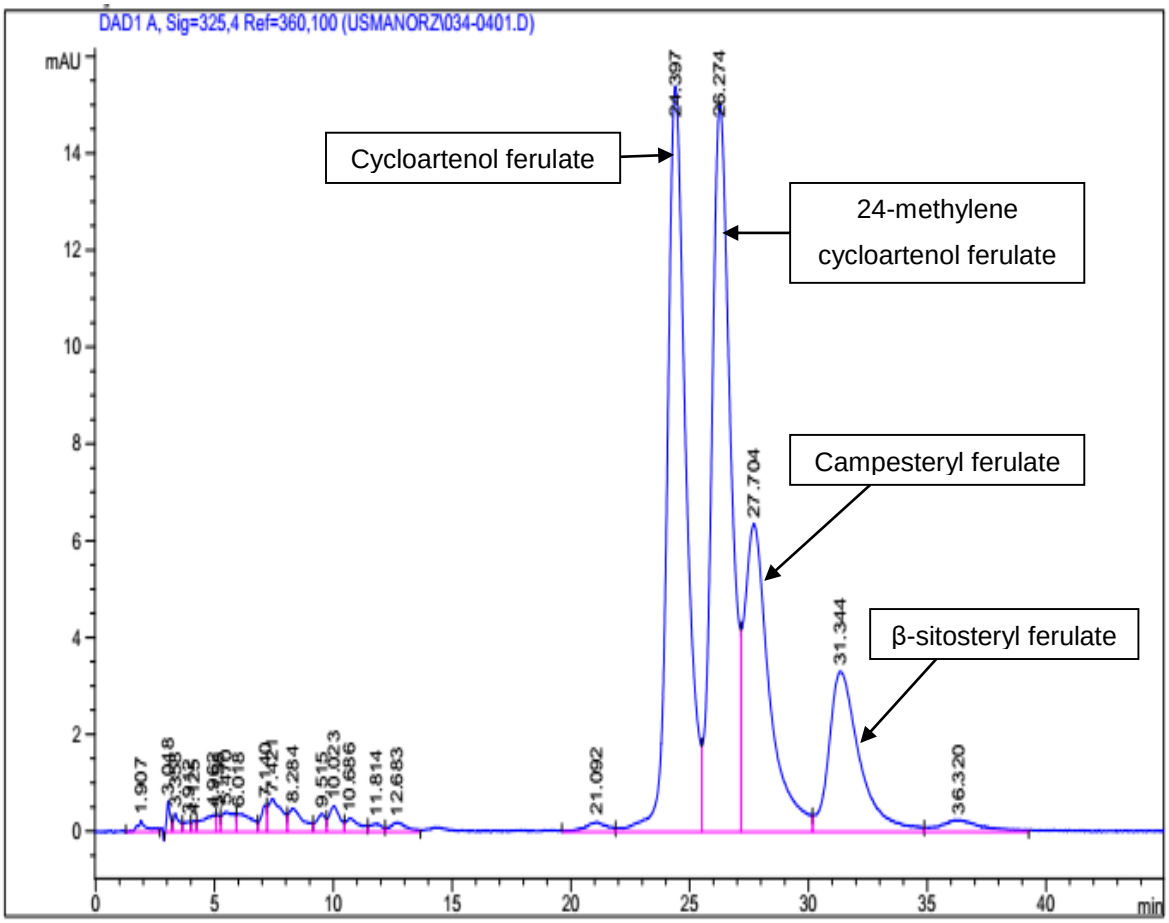
Annex 2. HPLC chromatograph for 40 mg/L standard GABA**.**



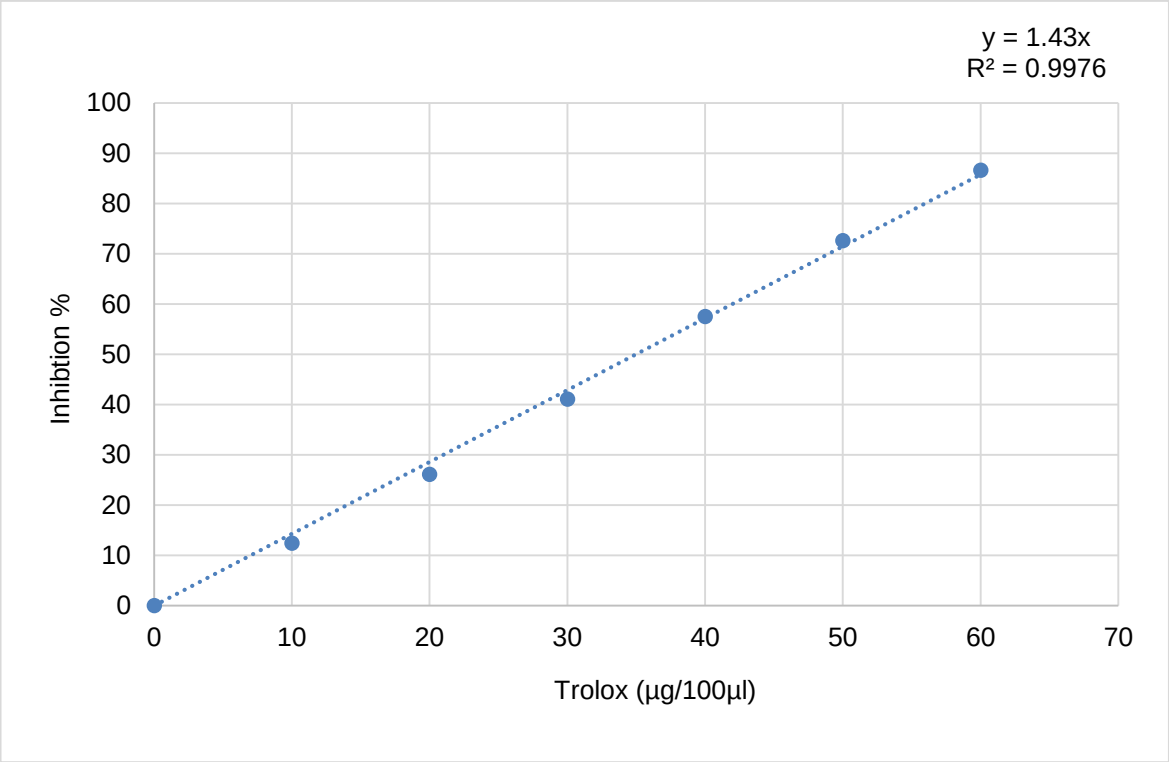
Annex 3. The slope of calibration for γ-oryzanol through HPLC**.**



Annex 4. HPLC chromatograph for 40 mg/L γ-oryzanol**.**



Annex 5. The slope of calibration for Trolox inhibition by Spectrophotometer**.**



Annex 6. Sensory evaluation scorecard for yogurt.

Name of the judge: _____

Date & Time: _____

Signature: _____

Parameters / **Samples**	**1**	**2**	**3**	
Appearance				
Taste / Flavor				
Color				
Overall acceptability				

Directions:

Samples will be evaluated for given sensory parameters and scored according to 9-point Hedonic scale.

The mouth will be rinsed each time with distilled water.

Hedonic Scale

9.	Like Extremely	**8.**	Like very much
7.	Like moderately	**6.**	Like slightly
5.	Neither like nor dislike	**4.**	Dislike Slightly
3.	Dislike moderately	**2.**	Dislike very much
1.	Dislike extremely		

CURRICULUM VITAE

Credentials

Name, Surname : Muhamamd Usman Akram

Place of Birth : Sialkot, Pakistan

Marital Status : Single

E-mail : mh.usman@hotmail.com

Address : College road, mohalla Fareed town, Daska, Sialkot, Pakistan.

Education

BSc. : BS (Hons) Dairy Technology (2009-2013)

MSc. : MSc Food Engineering (2015-2018)

Foreign Languages

English, Turkish and Urdu.

Work Experience

Areas of Experiences

Projects and Budgets

2016-2017 The Production of a Functional Yogurt Fortified with Brown Rice (Project No: FHD-2016-11950), Department of Food Engineering, Hacettepe University Ankara, 21,919.02 TL

Publications

Oral and Poster Presentation

HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
THESIS/DISSERTATION ORIGINALITY REPORT

HACETTEPE UNIVERSITY
GRADUATE SCHOOL OF SCIENCE AND ENGINEERING
TO THE DEPARTMENT OF FOOD ENGINEERING

Date: 12/02/2018

Thesis Title / Topic: **THE PRODUCTION OF A FUNCTIONAL YOGURT FORTIFIED WITH BROWN RICE**

According to the originality report obtained by myself/my thesis advisor by using the *Turnitin* plagiarism detection software and by applying the filtering options stated below on **12/02/2018** for the total of **70** pages including the a) Title Page, b) Introduction, c) Main Chapters, d) Conclusion sections of my thesis entitled as above, the similarity index of my thesis is **7%.**

Filtering options applied:

1. Bibliography/Works Cited **excluded**
2. Quotes **included**
3. Match size up to 5 words **excluded**

I declare that I have carefully read Hacettepe University Graduate School of Science and Engineering Guidelines for Obtaining and Using Thesis Originality Reports; that according to the maximum similarity index values specified in the Guidelines, my thesis does not include any form of plagiarism; that in any future detection of possible infringement of the regulations I accept all legal responsibility; and that all the information I have provided is correct to the best of my knowledge.

I respectfully submit this for approval.

12/02/2018
Date and Signature

Name Surname:	Muhammad Usman AKRAM
Student No:	N14148306
Department:	Department of Food Engineering
Program:	Food Engineering
Status:	☒ Masters ☐ Ph.D. ☐ Integrated Ph.D.

ADVISOR APPROVAL

APPROVED.

Prof. Dr. Ümran UYGUN
(Title, Name Surname, Signature)