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GRAPEFRUIT ALBEDO AND ITS SEED EFFECTS ON DIABETES

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Abstract

The *Diabetes mellitus* is one of the dominated disease and its focused studies are concerned to compete with this issue worldwide. Grapefruit albedo, seeds and their blends were supplemented in marmalade to evaluate their potential in controlling diabetes and developing immunity. The present study has been planned to find out the grapefruit effect on the *Diabetes mellitus* and immune system. Marmalades supplemented with higher albedo contents i.e. A_5 (containing 5% albedo powder) and A_4S_1 (containing 4% albedo and 1% seed powder) exhibited higher values for TPCs, TFCs, antioxidant activity as well as satisfactory acceptance by the consumers. These marmalades were further subjected to bio efficacy trials using Sprague Dawley rats induced with diabetes. Rats were divided into 3 groups *i.e.* G_0 (normal diet + C), G_1 (normal diet + A_5) and G_2 (normal diet + A_4S_1). The G1 and G2 exhibited less weight gain with significant decrease in all biomarkers of diabetes including blood glucose level, blood insulin level and HbA1c. Moreover, an increase in red blood cells (16% and 8% in G_1 and G_2, respectively), hemoglobin (11% and 8.57 %), white blood cells (17% and 6%) and platelet count (1% and 0.7%) was observed. Erythrocyte sedimentation rate and C-reactive protein were decreased by 15% and 5%, respectively, compared to the rats fed with the control diet. These results reflected the potential of grapefruit albedo and seeds in combating diabetes and developing immunity through the development of value-added food products.

Keywords: Grapefruit, Marmalade, Sprague Dawley Rats, Diabetes Biomarkers, Management

Highlights

- Waste materials of grapefruit (albedo and seeds) are ample source of phytochemicals.
- Albedo incorporation improve the organoleptic properties of product.
- Phytochemicals help in curing the diabetes and improve the inflammation status of diabetic patients.

INTRODUCTION

Pakistan is among the top ten citrus fruit-producing countries in the world. The citrus crop is the leading fruit crop worldwide. Around 124,246 thousand tonnes of citrus were produced in 2016 (FAO, 2016). China, Brazil, Spain, Mexico, India and the United States are major citrus-producing countries that contribute almost two third of global citrus production. Among citrus fruits, mandarins, lemon, oranges, mosambi and grapefruit are widely consumed (Liu, Heying, & Tanumihardjo, 2012). Grapefruit (*Citrus paradisi*) is the oldest citrus fruit known for its sweet and somewhat sour taste. It is grown mostly in tropical and subtropical areas. Grapefruits have several promoting benefits due to bioactive phytochemicals like organic acids, furanocoumarins, coumarins, carotenoids, flavonoids and polyphenols. A grapefruit has more than 188 mg potassium, which can fulfill the body's daily requirements and provides sufficient Vitamin C to improve the immune system's status. The high level of pectin and fiber found in citrus fruits may help to maintain healthy cholesterol and glucose levels (Faid, Fadlalla, & Khojah, 2017). White inner layer (albedo) and outer colored layer (flavedo) of grapefruit peels are rich in soluble and insoluble dietary fibers, which can be applied in the food industry for making the value added neutraceutical products (Khalil, Farghal, & Farag, 2020). On average, 34-50% of citrus fruits are processed into juices which produce large amounts of peels and seeds as waste (around 40 to 50% weight of fresh fruit), mostly used as cattle feed (Chavan, Singh, & Kaur, 2018; Zarate-Vilet, Gué, Delalonde, & Wisniewski, 2022).

Grapefruit peels are an ample source of potential nutraceutical components, including flavonoids (catechin, epigallocatechin, rutin, kampferol, flavonone, flavanone glycosides, polymethoxylated flavones, naringin, naringenin, limonin and quercetin) and phenolics (gallic acid, caffeic acid, ferulic acid, sinapinic acid, hesperidin and neohesperidin). These bioactive components vary from 0.5-20% dry basis in peels. Bioactive components in fresh and dry albedo exhibit pharmacological benefits such as anti-inflammatory, antimicrobial, antibacterial, anti-cancerous, and anti-oxidant and protect the body from multiple organ injuries (Ademosun et al., 2015; Scurria et al., 2021). These also inhibit prostate cancer and reduce the side effects of drugs used during oesophageal carcinoma chemotherapy (Nishad et al., 2020). Grapefruit is also a rich source of bioactive compounds such as vicenin-2, naringenin, rhoifolin, neohesperidin, hesperidin and didymin (Avula et al., 2016).

Almost 7.1 million people in Pakistan are suffering from diabetes, which is increasing daily. Around 46-75% Pakistani diabetic people have metabolic syndrome. Diabetic people are more prone to infections. The course of infection is also more complicated in diabetic patients. High blood sugar levels can weaken the immune system. Furthermore, some microorganisms become more virulent in high-glucose environments (Sarfraz, Sajid, & Ashraf, 2016). Secondary metabolites and bioactive components of grapefruit possess antidiabetic effects. Soluble and insoluble dietary fibers in grapefruit peels and seeds act as prebiotics and enhance the gut microbial activity that helps improve immunity status (Vazquez-Olivo, Gutiérrez-Grijalva, & Heredia, 2019).

Previously, the raw peel/seed and their extracts have been studied for their application against metabolic diseases, including oxidation stress (Huang, Liao, Qi, Jiang, & Yang, 2020; Sir Elkhatim, Elagib, & Hassan, 2018), obesity (Farouk, El-Sayeh, Mahmoud, & Sharaf, 2012), hyperchlostremia (Fayek et al., 2017), liver failure (Udom et al., 2018) and diabetes (Gan et al., 2020; Oyetayo, Akomolafe, & Oladapo, 2019). The current study

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comprises of development of grapefruit albedo and seed-based functional marmalade and its effects on diabetes and immunity.

MATERIALS AND METHODS

Grapefruits (pink variety) were procured from the local market of Faisalabad. The grapefruits were selected randomly based on certain quality attributes, including color uniformity, being free from abrasion, size and shape. For biological efficacy analysis, diagnostic kits were procured from Sigma-Aldrich bioassay and cayman chemicals (Cayman Europe, Estonia). All other chemicals and reagents were of analytical grade.

Preparation of albedo and seed powder: Grapefruits were peeled manually with the help of a knife. Peels, seeds and pulp were separated and kept in different pots. Albedo (white portion of peels) was separated from Flavedo. The albedo and seeds were dried in Hot Air Oven at 65 °C for 48 hours. After drying, they were subjected to milling by a rotor mill (ZM 200, Retsch GmbH, Haan, Germany). The resulting powders were packed in polyethylene bags for further use.

Preparation of marmalade: Grapefruit marmalade was prepared according to the method described by Tamer (2012) with some modifications. Different concentrations of albedo and seed powders were incorporated in marmalades, as given in table 1.

Table 1: Various concentrations of albedo and seed powders added to product

Treatment designation	Description
C	Control
A_5	Albedo powder (5%)
S_5	Seed powder (5%)
$A_{2.5}S_{2.5}$	Albedo powder (2.5%), Seed powder (2.5%)
A_1S_4	Albedo powder (1%), Seed powder (4%)
A_4S_1	Albedo powder (4%), Seed powder (1%)

***Characterization of marmalade*:**

Flavonoid contents, phenolic contents (Folin-Ciocalteu method) and antioxidant activity (DPPH method) of all marmalade samples were determined by the methods described by Chang et al. (2007), Sun et al. (2007) and Sreeramulu, Reddy, and Raghunath (2009), respectively described. The sensory quality of marmalades was evaluated by using the 9-point hedonic scale (9=like extremely, 1=dislike extremely) according to the method described by Lawless and Heymann (2010).

Bio-Efficacy trials: The treatments exhibiting better phytochemical and sensory characteristics were further subjected to *in vivo* trials using animal models. Bio-efficacy trials were performed using Sprague Dawley rats to explore the anti-hyperglycemic potential and functional worth of selected treatments. Purposely, experimental rats were acclimatized individually under optimum conditions (23±1 °C temperature and relative humidity 55-60%) in stainless steel cages. All animals were fed standard diet and tap water *ad libitum* (physiological saline) for seven days (one week) before experimentation.

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***Study plan on hyperglycemic rats*:** Rats (n=18) were randomly divided into 3 groups (G_0, G_1 and G_2). Streptozotocin injection (60 mg/kg) was administered intraperitoneally to induce diabetes in rats. G_0 was administered with control marmalade (C) whereas, G_1 and G_2 were fed on A_5 and A_4S_1 respectively, incorporated with a normal diet (Table 1). After 30 days, overnight fasted rats were weighed, slaughtered and their sera were collected to assess their diabetic and infection status.

***Calculations of feed and drink intake*:** During the entire study period, rats' feed and drink intake were calculated daily as described by Wolf and Weisbrode (2003) and Slemmer, Shaughnessy, Scanlan, Sweeney, and Gottschall-Pass (2012). The body weight and net feed intake were used to determine the feed efficiency ratio using the given mathematical relation.

$$FER\ (Feed\ Efficiency\ Ratio) = \frac{\text{Body weight Gain (g/day)}}{\text{Feed intake (g/day)}} \times 100$$

Biomarkers for Diabetes mellitus: Fasting blood glucose level (mg/dL) was determined through compatible strips employing the commercially available glucometer (OnCall ® Ez II; SN 303S0014E09) (DeMambro et al., 2010; Dong, Cai, Shen, & Liu, 2011). Blood insulin level was measured by Enzyme-Linked Immunosorbent Assay (ELISA Kit R&D system) (Ahn, Choi, Kim, & Ha, 2011). HbA1c levels were checked at baseline and the end of the study (30 days) (Slemmer et al., 2012).

Biomarkers for inflammation response evaluation: Hematological assay *i.e*. erythrocytes, leukocyte and thrombocyte indices were analyzed using Medonic M Series hematology analyzer (Boule Diagnostics Int. AB Stockholm, Sweden). Moreover, Erythrocyte Sedimentation Rate was assessed using Westergren's method using an automated ESR System (Gerard et al., 2015; Karbiner et al., 2013; Plebani & Piva, 2002; Uzun & Kalender, 2013) C-reactive protein was determined through the immunoturbidimetry method according to the procedure described in Putra, Saputro, Nurrahman, Herawati, and Dewi (2020).

Statistical analysis: A complete randomized design (2-factor factorial) was applied for animal study parameters and a simple complete randomized design was used for product characterization parameters using Statistix 8.1 model. Furthermore, Tukey's HSD multiple comparison test was applied for means comparison following the principles outlined by Montgomery (2017).

RESULTS AND DISSCUSION

Results

TFCs, TPCs and DPPH assay of marmalades: The mean values of total flavonoids, total phenols, and antioxidant activity of supplemented marmalades have been summarized in Table 2. The results show that the addition of albedo and seeds significantly affected the TPCs, TFCs and DPPH antioxidant activity of marmalades (p<0.05). All supplemented marmalades showed significant differences in mean values

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of TPCs and DPPH antioxidant activity. The minimum TPCs were observed in the control marmalade (121.3 mg GAE/100g) while maximum TPCs were observed in A_5 marmalade (222.6 mg GAE/100g). Similarly, minimum TFCs were observed in C (0.46 mg QE/100g) and maximum TFCs were recorded in A_5 (1.9 mg QE/100g). The antioxidant activity was decreased by decreasing the contents of albedo powder in treatments. Maximum antioxidant activity was exhibited by A_5 followed by A_4S_1, $A_{2.5}S_{2.5}$, A_1S_4, S_5 and C. Among all supplemented marmalades, samples containing higher albedo concentration had higher TPCs, TFCs and DPPH antioxidant (%) activity compared to marmalades having more seed proportion.

Table 2: Mean values of TFCs, TPCs and DPPH assay of supplemented marmalades with albedo and seeds

Phytochemistry	Treatments					
	C	A_5	S_5	$A_{2.5}S_{2.5}$	A_1S_4	A_4S_1
TPC (mg GAE/100g)	121.3 ± 0.5^{f}	222.6 ± 2.7^{a}	168.7 ± 3.3^{e}	186.4 ± 2.2^{c}	179.4 ± 1.4^{d}	201.3 ± 1.4^{b}
TFC (mg QE/100g)	0.46 ± 0.02^{d}	1.9 ± 0.02^{a}	0.98 ± 0.1^{c}	1.4 ± 0.04^{b}	1.37 ± 0.2^{b}	1.59 ± 0.08^{b}
DPPH (%)	12.13 ± 0.3^{f}	24.8 ± 0.4^{a}	14.6 ± 0.29^{e}	18.8 ± 0.3^{c}	16.8 ± 0.3^{d}	20.3 ± 0.7^{b}

Sensory evaluation: The addition of albedo and seed powders in marmalade significantly influenced the taste, color and texture. Maximum scores for taste, texture, aroma, appearance, overall acceptability and color were assigned to A_5 and A_4S_1. While other supplemented marmalades attained lower organoleptic scores. The sensory scores of organoleptic properties of all marmalades have been graphically represented in Figure 1.

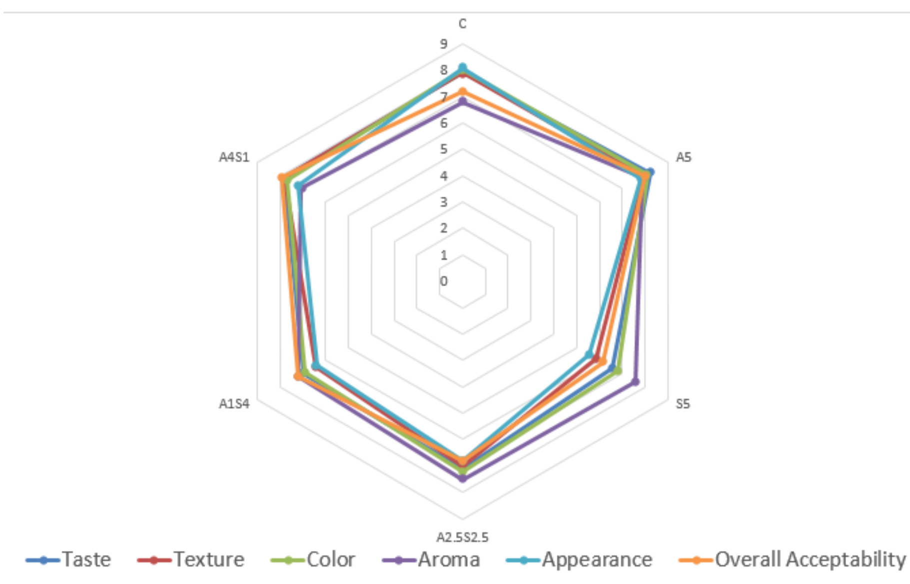

Figure 1: Sensory evaluation of supplemented marmalades with grapefruit albedo and seeds

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Calculations of feed and drink intake: The average weight gain, feed intake and water intake were higher in the control group compared to the treated groups (G_1 and G_2), as shown in Table 3. These symptoms are commonly known markers of type 2 diabetes due to insulin deficiency in diabetic rats. The average feed intake, water intake and weight gain were significantly reduced in rats treated with albedo and seed powders supplement. The average weight gain (g/day) in experimental groups *i.e.* G_0, G_1 and G_2 was 0.24, 0.112 and 0.208 (g/day), respectively. The rats fed with A5 showed lower feed intake, water intake and weight gain values compared to A4S1.

Table 3: Effect of supplemented marmalades on growth performance parameters

Experi-mental Groups	Growth Performance Parameters					
	Initial Body Weight (g)	Final Body Weight (g)	Weight gain (g/day)	Average Feed Intake (g/day)	Feed Efficiency Ratio (FER)	Average Water Intake (mL/day)
G_0	137.57±8.8^{c}	140.54±5.2^{c}	0.24±0.013^{a}	19.14±0.58^{a}	1.24±0.65^{a}	24.27±0.47^{a}
G_1	153.411±2.9^{a}	156.85±2.8^{a}	0.112±0.01^{c}	16.44±0.37^{c}	0.68±0.07^{a}	21.16±0.76^{c}
G_2	142.64±3.0^{b}	149.04±3.2^{b}	0.208±0.02^{b}	17.72±0.61^{b}	1.17±0.12^{a}	23.33±0.59^{b}

Values are expressed as mean±SD; one-way ANOVA followed by Tukey's HSD test ($p \leq 0.05$).

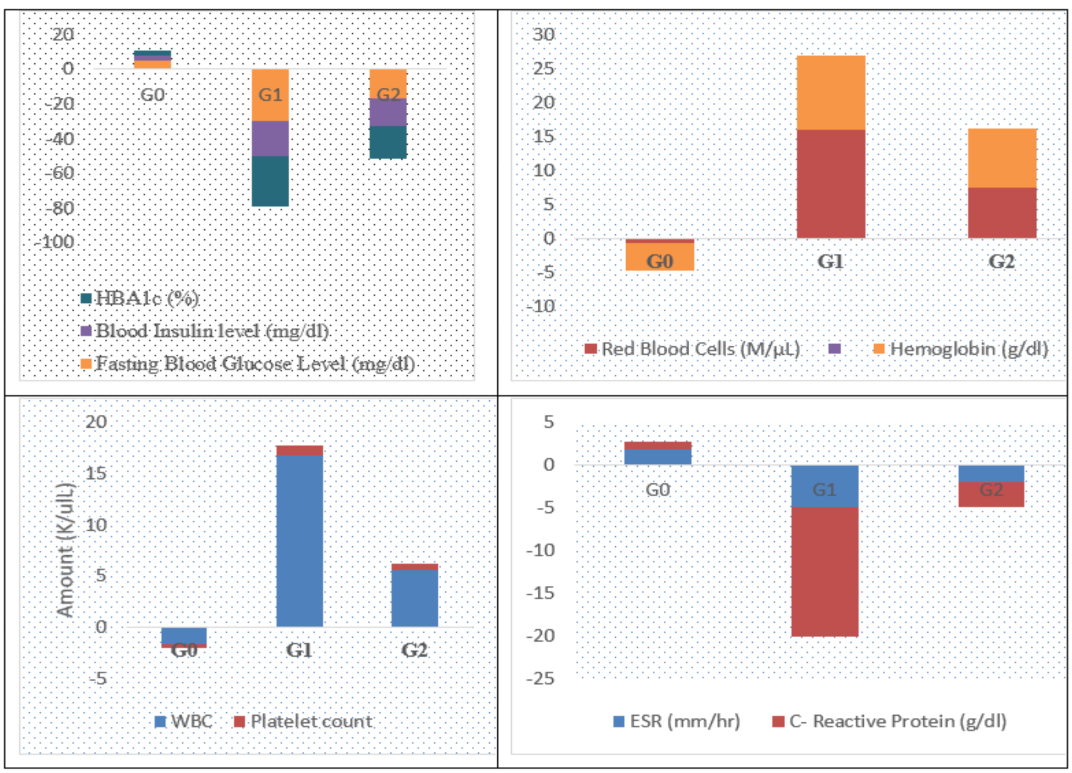

Figure 2: Graphical representation of effect of supplemented marmalades with albedo and seeds on biomarkers for *Diabetes mellitus* and immunity

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Biomarkers for Diabetes mellitus: It is evident from the results (shown in Table 4) that rats of G_0 had maximum fasting blood glucose level (261.6 mg/dl) followed by G_2 (194.8 mg/dl) and G_1 (182.8 mg/dl). Higher blood insulin level was observed in G_0 (35.98 units/l) followed by G_2 (26.5 units/l) and G_1 (23.14 units/l). Similarly, HbA1c was higher in G_0 (6.89 %) trailed by G_2 (4.68 %) and G_1 (4.39 %) at the end of the trial. The graphical representation of the results can be seen in figure 2. Fasting blood glucose level, blood insulin level and HbA1c were higher in the control group (G_0). In comparison, a significant decrease was observed in all biomarkers of *Diabetes mellitus* in G_1 and G_2 groups. These results indicate that the addition of grapefruit albedo and seeds in marmalade played a significant role in controlling diabetes. Moreover, the marmalades supplemented with albedo showed more antidiabetic potential than marmalades supplemented with seeds.

Table 4: Mean values of biomarkers for *Diabetes mellitus*

	Fasting Blood Glucose Level (mg/dl)		Biomarkers for *Diabetes mellitus*						
				Blood Insulin Level (units/l)			HbA1c (%)		
	Day (0)	**Day (30)**	**% Change**	**Day (0)**	**Day (30)**	**% Change**	**Day (0)**	**Day (30)**	**% Change**
G_0	249.54± 4.11[b]	261.6±6.8[a]	+4.83	34.9± 2.9[a]	35.98± 1.16[a]	+3.09	6.7± 0.4[a]	6.89± 0.31[a]	+2.8
G_1	260.43± 1.24[a]	182.8±5.4[e]	-30	28.9± 0.8[c]	23.14± 1.25[e]	-20	6.2± 0.14[b]	4.39± 0.05[f]	-29
G_2	233.7± 10.54[c]	194.8±3.9[d]	-17	31.6± 1.14[b]	26.5± 0.97[d]	-16	5.8± 0.1[c]	4.68± 0.1[e]	-19

Values are expressed as mean±SD; Two-way ANOVA followed by Tukey's HSD test ($p \leq 0.05$)

G_0 Normal diet+C

G_1 Normal diet+A_5

G_2 Normal diet+A_4S_1

C = Control; **A_5 =** Grapefruit Marmalade containing 5% albedo powder; A_4S_1**=** Grapefruit Marmalade containing 4% albedo and 1% seeds powder

-**ve=** Improvement **+ve=** No Improvement

Biomarkers for immune response evaluation: Effect of supplemented marmalades on erythrocyte indices, thrombocyte count, leukocyte count, inflammatory biomarkers, Erythrocyte Sedimentation Rate (ESR) and C-reactive protein have been represented in Tables 5, 6 and 7 respectively. The graphical representation of the results can be seen in figure 2. It is evident from the results that treatments significantly affected the red blood cells and hemoglobin in diabetic rats (p<0.05). Likewise, treatments significantly affected the white blood cells, platelet count and C-reactive protein (p<0.05). However, the effects of treatments on erythrocyte sedimentation rate in diabetic rats was non-significant. It was also observed that the combined effect of treatments and days significantly influenced the red blood cells, hemoglobin and white blood cells. Whereas, treatments and days

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interaction was non-significant in platelet count, erythrocyte sedimentation rate and C-reactive protein.

During the experiment, a maximum increase in total red blood cells was observed in G_1 (+16%) trailed by G_2 (+8%). However, a decrease in red blood cells was recorded in G_0 (-0.6%). Likewise, the highest increase in hemoglobin was observed in G_1 (+11%) followed by G_2 (+8.57%). However, a decrease in hemoglobin was recorded in G_0 (-4%). Similarly, a maximum increase in thrombocyte indices *i.e.* white blood cells was observed in G_1 (+17%) followed by G_2 (+6%); whereas, decrease in white blood cells was noted in G_0 (-2%). Control group showed decrease in platelet count (-0.37%). However, an increase in platelet count was observed in G_1 (+1%) and G_2 (564.68 to 568.79 K/µL; +0.7%).

The mean values of inflammatory biomarkers at base line and end of experiments showed that inflammation status in the control group (G_0) was increased. The erythrocyte sedimentation rate in G_0 was increased (+1.83%). G_1 and G_2 groups showed a significant decrease in erythrocyte sedimentation rate. A higher decrease in erythrocyte sedimentation rate was observed in G_1 (-5%) followed by G_2 (-2%). Similarly, the C-reactive protein level was increased in the control group (+0.91%). Whereas, the level of C- reactive protein was decreased in G_1 (-15%) and G_2 (-3%).

Table 5: Effect of marmalade diet on erythrocyte indices of experimental rats

Experimental groups	Erythrocyte indices					
	Red Blood cells (M/µL)			Hemoglobin (g/dl)		
	Day (0)	Day (30)	% Change	Day (0)	Day (30)	% Change
G_0	6.44±0.29^{de}	6.40±0.9^{e}	-0.6	11.5±0.67^{d}	11.05±1.5^{d}	-4
G_1	6.96±0.11^{c}	8.28±0.31^{a}	+16	12.54±0.39^{bc}	14.09±0.2^{a}	+11
G_2	6.87±0.2^{cd}	7.43±0.29^{b}	+8	11.85±0.62^{cd}	12.95±0.3^{b}	+8.57

Table 6: Effect of supplemented marmalade on WBCs and thrombocyte count

Experimental groups	White Blood cells (K/µL)			Platelet count (K/µL)		
	Day (0)	Day (30)	% Change	Day (0)	Day (30)	% Change
G_0	8.12±0.43^{b}	7.99±1.12^{b}	-2	548.51±11.3^{b}	546.47±14.61^{b}	-0.37
G_1	8.5±0.2^{b}	10.2±0.5^{a}	+17	567.78±3.55^{a}	573.58±3.98^{a}	+1
G_2	8.17±10.54^{b}	8.65±0.66^{b}	+6	564.68±10.3^{a}	568.79±10.6^{a}	+0.7

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Table 7: Effect of supplemented marmalades with albedo and seeds on inflammatory biomarkers

Experimental groups	Inflammatory Biomarkers					
	ESR (Erythrocyte Sedimentation Rate) mm/hr			C-reactive Protein (g/dl)		
	Day (0)	Day (30)	% Change	Day (0)	Day (30)	% Change
G_0	5.696±0.25[b]	5.8±0.32[a]	+1.83	7.68±0.42[a]	7.75±0.8[ab]	+0.91
G_1	5.723±0.15[a]	5.42±0.29[e]	-5	7.39±0.4[c]	6.26±0.37[e]	-15
G_2	5.729±0.14[c]	5.60±0.11[d]	-2	7.6±0.7[b]	7.4±0.65[d]	-3

Values are expressed as mean±SD; Two-way ANOVA followed by Tukey's HSD test ($p \leq 0.05$)

G_0 Normal diet+C

G_1 Normal diet+A_5

G_2 Normal diet+A_4S_1

C = Control; **A_5 =** Grapefruit Marmalade containing 5% albedo powder; A_4S_1**=** Grapefruit Marmalade containing 4% albedo and 1% seeds powder

-**ve=** Decreasing (Improvement) **+ve=** Increasing (No improvement)

DISCUSSION

The mean values of total flavonoids, total phenols, and antioxidant activity of supplemented marmalades have been summarized in Table 2. The results show that the addition of albedo and seeds significantly affected the TPCs, TFCs and DPPH antioxidant activity of marmalades (p<0.05). All supplemented marmalades showed significant differences in mean values of TPCs and DPPH antioxidant activity. Estaji, Mohammadi-Moghaddam, Gholizade-Eshan, Firoozzare, and Hooshmand-Dalir (2020) studied the effect of plum peel on the bioactivity of plum marmalade. Authors reported that phenolic compounds (38.65-212.69 mg GAE/100g) and antioxidant activity (33.65-85.47%) in plum marmalade were improved as the concentration of plum peels was increased. Additionally, Ben-Rejeb, Dhen, Kassebi, and Gargouri (2020) prepared the grapefruit jelly and reported that grapefruit jelly had a significant amount of total phenols (123.16 mg GAE/100g), total flavonoids (1.54 mg QE/100g) and exhibited high antioxidant activity. Another study conducted research on watermelon candies fortified with different concentrations of orange albedo and flavedo. It was concluded that fortified candy formulations had higher TPC, TFC and antioxidant activity (Marinelli et al., 2020).

Among all supplemented marmalades, samples containing higher albedo concentration had higher TPCs, TFCs and DPPH antioxidant (%) activity compared to marmalades having more seed proportion. This observation could be due to the presence of more TPCs, TFCs and DPPH antioxidant (%) activity in grapefruit albedo as compared to seeds, as also reported by Assefa and Keum (2017); Sir Elkhatim et al. (2018) and Wang et al. (2020).

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Sensory response significantly influences product demand, consumer acceptability and purchase intent. Quality of food products is examined by complex sensation resulting from the interaction of human senses *i.e.* smell, touch, taste, etc. (Ben-Rejeb et al., 2020). The addition of albedo and seed powders in marmalade significantly influenced the taste, color and texture. The more concentration of seeds powder reduced the appearance, aroma, color, taste and overall acceptance of marmalade. This could be due to compounds in seeds powder, including poncirin, hesperidin, polymethyoxylated flavones and non-hesperidin which are mainly responsible for the taste change (Kiefl et al., 2017).

Similar results were reported by Teixeira et al. (2020) in which orange jam containing 4% orange peels was reported to be highly acceptable by consumers as compared to other treatments containing 8 and 12% peels proportion. In another study, Younis, Islam, Jahan, Yousuf, and Ray (2015) prepared the papaya jams by incorporating the 2.5, 5, 7.5, 10 and 12.5% mosambi peels. The authors reported that papaya jam having 5% mosambi peels was more acceptable by consumers. Present results are also by the findings of Levent (2014) who incorporated 5% lemon, orange and grapefruit albedo in tarhana soup and reported acceptable sensory response. Other reports on sensory response similar to present results can be found in Marinelli et al. (2020), Özbek, Şahin-Yeşilçubuk, and Demirel (2019), Pérez-Herrera, Martínez-Gutiérrez, León-Martínez, and Sánchez-Medina (2020) and Hussein, Kamil, Hegazy, Mahmoud, and Ibrahim (2015). These symptoms are commonly known markers of type 2 diabetes due to insulin deficiency in diabetic rats. This effect could be due to the higher amount of fibers in the albedo as compared to seeds (Teixeira et al., 2020).

Zhang et al. (2020) described that the anti-obesity potential of citrus peels could be due to dynamic changes in gut microbiota and the improvement of biological processes of metabolism. It significantly increases the amount of fecal short-chain fatty acids *i.e.* acetic acid (43%) and propionic acid (86%). It also decreases the prevalence of proteobacteria and the amount of firmicutes to bacteroidetes ratio, which significantly affects the obesity pathway. These results were similar to the findings of Oyedemi, Adewusi, Aiyegoro, and Akinpelu (2011) who elucidated the antidiabetic effect of *Afzelia Africana* (mahogany plant) in controlling the desire for water and feed intake under diabetic conditions. Similar findings were reported by Kang, Song, Lee, Chang, and Lee (2018). The authors elucidated the significant decrease in weight, BMI, triglycerides, cholesterol and low-density lipid profile in 118 patients taking citrus (*Citrus unshiu*) peel pellets for 4 weeks.

Type 2 diabetes is a complicated metabolic disorder denoted by glucose intolerance and hyperglycemia. Increasing evidence from animal models and cells suggests that bioactive components present in peels and seeds may directly ameliorate hyperglycemia, preventing amyloid aggregation and enhancing the insulin secretion by pancreatic β cells (Li, Zhang, Rasool, Geetha, & Babu, 2019). The present study's results corroborate the findings of Oboh, Olasehinde, and Ademosun (2017). The authors expounded the antidiabetic effect of essential oils extracted from peels of orange and lemon. The results revealed the significant inhibitory effect of essential oils on α-amylase and α-glucosidase. Comparable findings were also documented by Ali et al. (2020). The authors elucidated

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the antidiabetic, anti-hyperlipidemia and antioxidant potency of fruit peels of *Citrus reticulata* in nicotinamide /streptozotocin-induced type 2 diabetes Wister rats. According to Gan et al. (2020), *in-vitro* digestive glucose rate of bread incorporated with soluble dietary fibers of grapefruit peels was decreased significantly. In another study, Ashraf, Butt, Iqbal, and Suleria (2017) investigated the hypoglycemic potential of citrus (orange) peel powder through rodent modeling. It was concluded that the nutraceutical diet alleviated serum glucose by 8.96% and enhanced insulin secretion by 5.41% in rodents. In another study, serum glucose level was decreased significantly in fish by feeding them *Citrus bergamia* peel oil along with a normal diet (Kesbiç, Acar, Yilmaz, & Aydin, 2020). The mechanism of grapefruit waste in controlling diabetes includes the upregulation of insulin secretion capability as well as intracellular insulin concentration in RIN-m5F cells and Glut4 in HepG2 cells (Lin, Huang, Chen, & Peng, 2021). Pectin obtained from grapefruit peel and other citrus waste showed inhibition of enzymes (against α-glucosidase, pancreatic cholesterol esterase and lipase) linked to managing hyperglycemia and hyperlipidemia and hypercholesterolemia. α-glucosidase is responsible for the hydrolysis of glucose in the body, which prevents the postprandial glucose peaks from playing a key role in the management of type- 2 diabetes (Picot-Allain et al., 2022).

It is evident from the results that treatments significantly affected the red blood cells and hemoglobin in diabetic rats ($p<0.05$). Whereas, treatments and days interaction was non-significant in platelet count, erythrocyte sedimentation rate and C-reactive protein. The present findings are in harmony with the findings of Tsujiyama, Mubassara, Aoshima, and Hossain (2013) who reported that aqueous peel extract of grapefruit suppressed the inflammation in mice cavities. The peel extract (0.1 mL/10g) was administered orally in mice. The anti-inflammatory action of grapefruit peel extract was comparable to indomethacin (anti-inflammatory drug) in mice. Hence, grapefruit peel extract could be utilized to develop anti-inflammatory supplements and foods. Another study investigated the chemical and pharmacological potential of citrus peel oils through *in vitro* and *in vivo* analysis. It was revealed that Citrus limetta peels inhibited the production of pro-inflammatory cytokines, lipid peroxidation, C-reactive protein, ameliorate the histological damage and reactive oxygen species production in white rabbits and mice (experimental animals). *In vitro* and *in vivo* analysis of citrus peels showed that they are safe for topical application on the skin (Maurya, Mohanty, Pal, Chanotiya, & Bawankule, 2018). The current study's results align with earlier investigations of Jiang et al. (2019), who studied the anti-inflammatory and hepatoprotective effects of citrus peels in rats. It was reported that citrus peel extract suppressed the activities of phosphorylated cytokines (by inhibiting the systemic and intrahepatic inflammation), significantly attenuated the hepatic lesions and decreased the non-alcoholic fatty liver disease activity scores. It was documented by Ashraf et al. (2017) that red blood cells, white blood cells and platelet count in rodents were increased by the intake of orange peel powder and orange peel extract with time.

Adding albedo and seeds improved the hematological parameters (red blood cells, hemoglobin, platelet count and white blood cells). This effect was recorded due to the presence of phenolic compounds (polyphenols, hydrocarbons, terpenes), which could

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inhibit the hemolysis of red blood cells and lipid peroxidation of cells in diabetic rats (Soji-Omoniwa, Muhammad, Omoniwa, & Usman, 2014). Flavonoids (hesperidin and naringin) present in albedo and seeds stimulate erythropoietin secretion, enhancing the production of red blood cells (Mahmoud, 2013). The platelet count in diabetic-treated rats was improved markedly. This observation indicated albedo and seeds' ability to stimulate the clotting factors' synthesis (Oyedemi et al., 2011). Thus, the current study revealed that albedo and seeds could prevent -associated diabetes inflammation by attenuating the hematological parameters, erythrocyte sedimentation rate and C - reactive protein levels in diabetic rats.

CONCLUSION

Using underutilized parts of fruits in foods can reduce the negative impact of organic waste disposal on the environment and promote the consumer access to healthier foods. The present study evaluated the nutritional benefits of grapefruit albedo and seeds in controlling *Diabetes mellitus* and inflammation. The study concluded that albedo has more phenolic contents, flavonoids and antioxidants than seeds. Outcomes of sensory analysis and efficacy study proved that the use of albedo powder supplemented marmalade (A5) and seeds (A_4S_1) is efficient against diabetes as well as inflammation. However, the addition of 5% seeds powder reduced the consumer acceptability of marmalade as well as proved less effective against diabetes as compared to albedo. Supplemented marmalade effectively controlled diabetes by improving the hematological and inflammation parameters.

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Conflict of Interest

All authors declare that there are no conflict of interest.

Ethical statement

The ethical committee of the University of Agriculture Faisalabad-Pakistan approved the study.

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