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3 **Bioevaluation of agro-waste for postprandial glucose and insulin**
4 **concentration in blood**

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12
13 **Abstract**

14 **Objective:** To evaluate the effect of cookies supplemented with apple pomace
15 and mango-peel powder on postprandial glucose and insulin concentration.

16 **Method:** The experimental study was conducted from February to August,
17 2018, at the Nutrition Counselling Centre, Government College Women
18 University, Faisalabad, Pakistan. Different cookies with apple pomace and
19 mango-peel powder were prepared and the most nutritive acceptable cookies
20 were used to determine their postprandial effect on glucose and insulin
21 concentrations against the control cookies made with white flour only in female
22 subjects. Adult women were selected through advertisement with normal body
23 mass index. Data was analysed using SPSS 17.

24 **Results:** All the 30 subjects received enriched and control cookies at different
25 time slots during the study. The overall mean age of the sample was 25±10
26 years and each subject had body mass index <25kg/m². Overall blood glucose
27 and insulin concentrations were significantly lower with treatment cookies ,
28 compared to the control cookies (p<0.05).

29 **Conclusion:** It was evident that fruit processing waste can be used as a
30 nutraceutical agent in diet-based modules.

31 **Key Words:** Glycemic response, Type 2 diabetes mellitus, Insulin control.
32

33 **Introduction**

34 Postprandial hyperglycaemia (PPHG) is linked with the concentration and rate
35 of glucose absorption due to starch intake in the diet¹. PPHG and
36 hyperinsulinaemia (HI) are early detectors of various cardiovascular diseases
37 (CVDs) and type 2 diabetes mellitus (T2DM)². Studies have demonstrated that
38 dietary fibre and some phytochemicals may play significant role in the
39 reduction of postprandial glycaemia and insulinaemia. Polyphenols present
40 abundantly in plant food show their efficacy against PPGH by interfering with
41 the metabolic enzymes present in the intestine³. Moreover, the plant dietary
42 fibre hinders the transport of glucose into the blood by decreasing its digestion
43 and absorption through intestinal wall⁴. Among the plant-derived economical
44 and abundantly available indigenous resources, the study on apple pomace and
45 mango-peel has demonstrated that these by-products of fruit processing
46 industries are marvellous sources for both high-quality dietary fibres and
47 bioactive compounds⁵. These can readily be used for the improvement of
48 postprandial glucose and insulin concentration in healthy subjects. Furthermore,
49 in Pakistan the production of apples is about 598,804 tons, while mango
50 occupies 14% of the total cultivated area for fruits and is the second largest fruit
51 grown after citrus⁶. The estimated capacity for juice production is almost
52 400,000 Mt and processing results in wastage of valuable material⁷.

53 Further, cookies are considered superior products for supplementation due to
54 higher acceptance by community, better sensory acceptability and longer shelf-
55 life⁸. The current study was planned to evaluate the hypoglycaemic potential of
56 apple pomace enriched cookies (APECs) and mango-peel enriched cookies
57 (MPECs) to assess postprandial glycaemic response in humans.

58

59 **Materials and Methods**

60 The experimental study was conducted from February to August, 2018, at the
61 Nutrition Counselling Centre, Government College Women University, Faisalabad,
62 Pakistan. After approval from the institutional ethics review committee, the sample
63 was raised through advertisement posted and distributed at different clinics of
64 the city. Written informed consent was obtained from all the subjects. Those
65 included were adult females with normal body mass index (BMI). Those having
66 obesity, diabetes, hypertension or allergic reaction to any of the contents were
67 excluded.

68 Apple pomace and mango peels were obtained from the local fruit processing
69 industry. Apple pomace was blanched, dried (60°C) and was ground to a
70 particle size of 500–600µm. The same procedure was adopted for mango peels⁵.
71 Different levels of apple pomace powder (APP) and mango-peel powder (MPP)
72 were supplemented in white flour (WF) (5-25g/100g of WF) to prepare the
73 cookies using the method as described by the American Association for Clinical
74 Chemistry (AACC)⁹. The supplemented cookies were analysed for various
75 sensory properties, like colour, flavour, taste, texture, crispness and overall
76 acceptability, against the control cookies that had 0% level of supplementation.
77 The comparison was made by a panel of experts using 9-point Hedonic Score
78 System¹⁰. The best found cookies were used subsequently.

79 The subjects were given different cookies as control (C), APEC and MPEC
80 groups in different sessions with a washout period of 10 days between each
81 treatment. The subjects were provided the food frequency and discomfort
82 questionnaire to record any discomfort¹¹.

83 The volunteers were offered cookies (50g) in fasting state of at least 12 hours
84 along with one glass of plain water. The blood was drawn for glucose and
85 insulin concentration at baseline, 30, 60, 120 and 180 minutes post-ingestion.
86 The blood was tested for glucose concentration using glucometer and venous

87 blood (2mL) was collected in tubes, allowed to stand till clot was formed and
88 was centrifuged to get clear serum sample. The serum was analysed for insulin
89 concentration following enzyme-linked immunosorbent assay (ELISA) kit
90 protocol (immune-reactive insulin by double antibody technique) using ELISA
91 plate reader¹².

92 It was a single-blind study where the subjects were not informed about the type
93 of cookies they were consuming. The same subjects acted as control and
94 treatment groups at different sessions with washout period of 10 days. Data
95 was analysed using SPSS 17. Analysis of variance (ANOVA) was done to see
96 the level of significance. For post-hoc test, the least significant difference was
97 calculated at $p \leq 0.05$.

98

99 **Results**

100 All the 30 subjects received enriched and control cookies at different time slots
101 during the study. The overall mean age of the sample was 25 ± 10 years and each
102 subject had BMI $< 25 \text{ kg/m}^2$. Sensory evaluation of control and supplemented
103 cookies were significantly different ($p \leq 0.05$) (Figure). The best selected cookies
104 were used for further analyses (Table 1).

105 The mean values for blood glucose concentration showed a significant
106 difference between the patterns of glucose concentrations at different time
107 intervals ($p < 0.05$) (Table 2).

108 Overall insulin concentration was highest at 30 minutes which fell down with
109 time (Table 3).

110

111 **Discussion**

112 The findings showed that the dietary intervention in the form of cookies proved
113 to be a better strategy to improve fibre and phytochemical content of daily diet.

114 A study ¹³ also observed sensory properties of orange pulp and peel powder
115 supplemented cookies and found these acceptable up to 15%. Above this level,

116 the cookies gave the feeling of dryness and became difficult to swallow without
117 water. In the present study, with increase in the level of supplementation the
118 texture of cookies became hard and the shape was also not up to the mark due to
119 higher content of fibre. Similarly, a study¹⁴ described that cookies supplemented
120 with mango/cassava/soybean composite flours were more acceptable compared
121 to wheat flour cookies.

122 Studies have shown that increase in postprandial glucose concentration results
123 in the development of T2DM and CVDs¹⁵⁻¹⁷. The results of blood glucose and
124 insulin concentrations clearly showed that glycaemic response decreased
125 significantly with the addition of MPP as well as APP compared to the control
126 cookies. This may be attributed to higher dietary fibre content in APP and
127 MPP⁵. Dietary fibre is well known for decreasing postprandial glycaemia and
128 insulinaemia because it delays and slows down glucose absorption and in return
129 the level of insulin is also regulated¹⁶. Also, the consumption of dietary fibre has
130 an inverse relation with glycaemic load, and repeated higher glycaemic load
131 may lead towards the incidence of T2DM and insulin resistance (IR). Similar
132 results were also demonstrated for cookies made with the addition of resistant
133 starch¹⁷ and those supplemented with viscous fibre¹⁸. Moreover, it is well
134 established that a low-glycaemic index diet may positively change β -cell
135 function, coagulation ability and lipid profile¹⁹.

136

137 **Conclusion**

138 The waste from the fruit processing industry can be successfully used for the
139 preparation of cookies which were liked by the consumers even more than the
140 control cookies without supplementation. The supplemented cookies managed
141 well postprandial glucose and insulin concentrations compared to the control
142 cookies.

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148 has been changed to “Institute of Home and Food Sciences, Govt. College
149 University, Faisalabad, Pakistan”.

150 **Conflict of Interest:** None.

151 **Source of Funding:** None.

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 217 **Table 1: Composition of selected cookies for postprandial trial**

Parameter	Control Cookies	Apple Pomace Powder Enriched Cookies (APEC)	Mango-Peel Powder Enriched Cookies (MPEC)
Portion Weight (g)	50.00	50.00	50.00
Energy (Kcal)	247.47	219.82	218.99
Total Carbohydrates (g)	35.00	35.00	35.00
Available Carbohydrates (g)	33.70	28.65	28.89
Total Dietary Fibre (g)	1.30	6.35	6.11
Fat (g)	10.43	9.78	9.63
Protein (g)	4.70	4.30	4.23

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222 **Table 2: Effect of different treatments on blood glucose concentration**
 223 **(mg/dL±SEM) at various time spans**

Treatments	Baseline	30min	60min	120min	180min	Overall Means
Control	96.67±0.88	145.00±1.15 ^a	134.33±1.15 ^a	122.32±2.02 ^a	101.00±0.57 ^a	119.87±5.02 ^A
APEC	96.69±1.20	126.34±1.76 ^b	110.68±1.76 ^b	103.00±1.15 ^b	98.00±0.58 ^b	106.93±2.59 ^B
MPEC	96.68±0.89	120.67±0.88 ^c	104.63±1.45 ^c	98.67±0.89 ^b	97.00±0.57 ^b	103.53±2.44 ^B
Overall Means	97.67±0.50 ^D	130.67±3.37 ^A	116.56±4.50 ^B	108.00±3.71 ^C	98.67±0.67 ^D	

224 ^{a-c} different letters in a column show significant difference at $p \leq 0.05$
 225 ^{AB} different letters in a column show significant difference at $p \leq 0.05$
 226 ^{A-D} different letters in a column show significant difference at $p \leq 0.05$
 227 APEC: Apple pomace enriched cookies
 228 MPEC: Mango-peel enriched cookies
 229 SEM: Standard error of mean.

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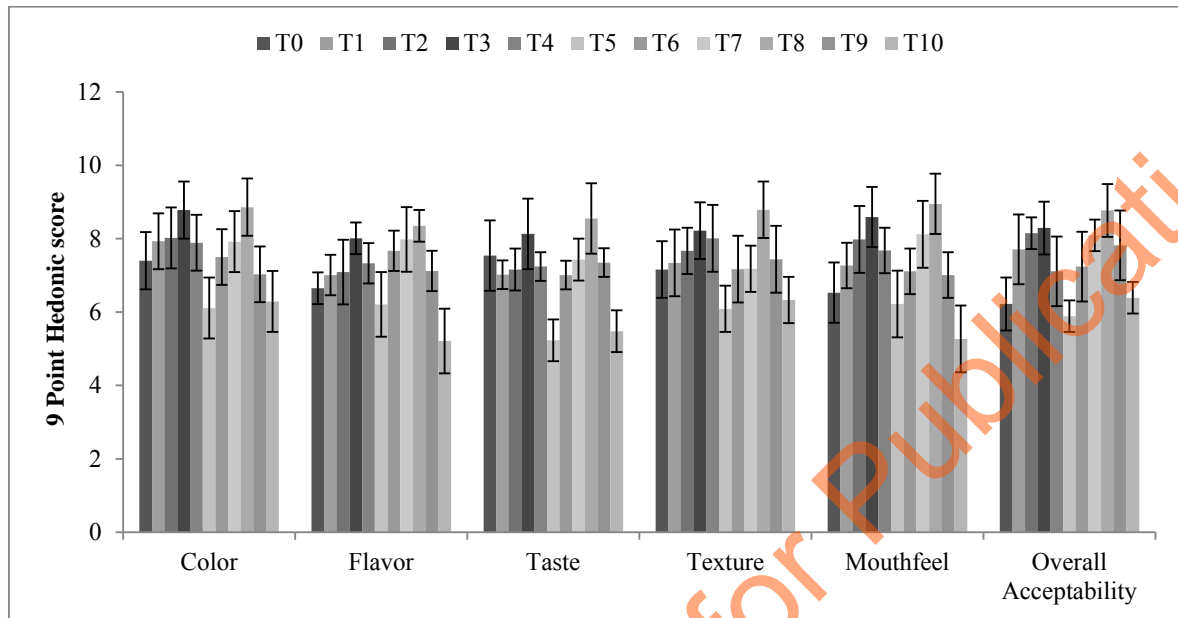
233 **Table 3: Effect of different treatments on blood insulin concentration**
 234 **(pmol/L±SEM) at various time spans**

Treatments	Baseline	30min	60min	120min	180min	Overall Mean
Control	85.00±1.52	357.67±0.88 ^a	226.67±0.87 ^a	168.00±1.53 ^a	95.00±1.52 ^a	186.47±26.47 ^A
APEC	85.00±1.67	223.34±1.45 ^b	205.67±33.15 ^b	127.00±1.15 ^b	88.34±1.2 ^b	145.86±16.54 ^B
MPEC	84.35±1.86	205.33±0.88 ^c	162.29±0.86 ^c	113.33±4.17 ^c	87.00±0.57 ^b	130.48±12.52 ^B
Overall Means	84.77±0.83 ^D	262.11±24.03 ^A	198.22±13.47 ^B	136.11±8.3 ^C	90.11±1.3 ^D	

235 ^{a-c} different letters in a column show significant difference at $p \leq 0.05$
 236 ^{AB} different letters in a column show significant difference at $p \leq 0.05$
 237 ^{A-D} different letters in a column show significant difference at $p \leq 0.05$
 238 APEC: Apple pomace enriched cookies
 239 MPEC: Mango-peel enriched cookie
 240 SEM: Standard error of mean.

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Figure: Sensory evaluation score (\pm SEM) of apple pomace and mango-peel cookies to get the best accepted cookies for further use. The values are means of 10 replicates along with standard error of mean (SEM).